[illegible] RESEARCH BOARD
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[illegible] Juvenile salmon [illegible] and some results [illegible]

[illegible] density of [illegible] Bear Lake [illegible] feeding on [illegible] cod [illegible]

[illegible] Use of [illegible] exposed to a series of light [illegible]

[illegible] commercial Atlantic [illegible]

[illegible] Responses [illegible] Salmo [illegible]

[illegible] Lipids of cod [illegible]

[illegible] rate of [illegible] embryos [illegible] or rupture of [illegible]

[illegible] distribution of [illegible]

[illegible] J. Dyer, and [illegible] of cod fillets after single [illegible] double [illegible]

Notes

[illegible] trawl catches. By A. W. [illegible]

[illegible] of salmon scales [illegible]

[illegible] (Oncorhynchus [illegible]

[illegible] By T. K. [illegible] Lake Ainslie, [illegible]

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Juvenile Salmon in the Estuary and Lower Nabisipi River and Some Results of Tagging[1,2]

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ABSTRACT

Morphometric and hydrographic conditions indicated Nabisipi River, Quebec, has a fjord-type estuary with warm surface water of low salinity flowing out over cold saline water from the Gulf of St. Lawrence at all phases of the tidal cycle. The age, weight, and condition factors of 231 juvenile salmon from the estuary and 200 from the lower reaches of the river are given. Ages ranged from 1+ to 4+, condition factors (100 × weight in grams divided by the length in centimeters cubed) were slightly below 1.0 in the river and slightly higher in the estuary. Amphipods and capelin eggs were important constituents in the diet in the estuary, Corixidae in the river. Tagging indicated a growth rate of about 0.8 mm/day in the estuary. Both tagging and the handling of marked fish retarded growth. Tagging retarded growth for approximately 20 days. Tagging returns indicated a population of between 700 and 2500 fish near the mouth of the estuary in June and July 1961. These were thought to be remnants of the spring smolt run. In addition there was a small resident population of salmon parr living in the estuary. Numbers of fish in the estuary changed considerably from year to year.

SOMMAIRE

Selon les relevés morphométriques et hydrographiques, la rivière Nabisipi possède un estuaire en forme de fjord dont les eaux de surface, chaudes et de basse salinité, s'écoulent en tout temps de la marée audessus des eaux froides et salines du golfe Saint-Laurent. La détermination de l'âge, du poids et du coefficient de condition de jeunes saumons capturés dans l'estuaire (231) et dans la partie basse de la rivière (200) révèlent que l'âge varie de 1+ à 4+ et que les coefficients de condition sont légèrement plus bas que 1.0 dans la rivière et légèrement plus haut dans l'estuaire. Dans l'estuaire, amphipodes et oeufs de capelan constituent une part importante du régime alimentaire, alors que dans la rivière ce sont les Corixidae. Dans l'estuaire, l'étiquetage indique un taux de croissance de 0.8 mm par jour. L'étiquetage des jeunes saumons et leur manipulation subséquente influent sur leur croissance. L'étiquetage retarde la croissance pendant une vingtaine de jours. En juin et juillet 1961, les recaptures de poissons étiquetés indiquent une population variant entre 700 et 2500 individus. On croit que ces poissons faisaient partie de la migration printanière des saumonneaux. De plus, il y a une petite population de jeunes saumons vivant dans l'estuaire. Le nombre de poissons à cet endroit change considérablement d'année en année.

INTRODUCTION

IN 1960 the Quebec Department of Fish and Game established a research station in the estuary of the Nabisipi River which flows into the north shore of the Gulf of St. Lawrence about 180 miles east of Sept-Iles (Bourassa, 1963).

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[2] Department of Tourism, Fish and Game, Quebec, Bulletin No. 9, Wildlife Service.

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The river is about 100 miles long and has a mean annual rate of discharge of about 2750 ft^3/sec. The purpose of the station is to find means of improving stocks of Atlantic salmon (*Salmo salar* L.) in north shore rivers. Preliminary observations in 1960 indicated there was a large population of rapidly growing salmon parr in the estuary and these fish were felt to be important because they might influence the salmon producing capacity of the river. Work was undertaken in 1961 to obtain data on the age and growth of parr living in the estuary. In addition information was gathered on sex ratios and feeding habits. A tagging program was started in 1961 and continued in 1962. In this report the preliminary results of the 1961 tagging only are considered, because these are pertinent to a consideration of the status of stocks of salmon parr present in the estuary that year.

MATERIAL AND METHODS

DESCRIPTION OF THE ESTUARY

The estuary is 9000 ft long and averages 425 ft wide. There is a 40-ft waterfall at its head and the other extremity is marked by a bar in the river mouth over which there is a maximum depth of 6.5 ft at mean water level. The channel is relatively deep, with steep rocky sides except in a few sheltered places and at the mouth where there are sand beaches. Depths of over 10 ft are commonly encountered within 25 ft of the shore. Figure 1 shows estuary with depth countours, at mean water level, in 5-ft intervals. For clarity the east–west scale of the chart is twice that of the north–south. The bed of the estuary is composed largely of bare rock and sand. Mud and gravel are present in some places. Rooted aquatic vegetation (*Potamogeton* sp.) is restricted to a few shallow areas.

There is a well-marked thermal and chemical stratification of water in the estuary. Stratification remains stable throughout the tidal cycle and probably over a wide range of river discharge. While the tide is rising a wedge of cold, highly saline water enters the estuary by flowing over the bar in the river mouth and penetrates to its head. During the summer the temperature of this water varies from 3 to 9 C. The salinity is between 20 and 28‰. Above this is a layer of water approximately 5 ft thick which separates a surface wedge of warm brackish river water from the underlying sea water. The change in salinity across this discontinuity layer is at least 15‰ while the variation in temperature in summer exceeds 5 C degrees. The upper wedge of water has a salinity between 1 and 6‰ and a temperature of 15–17 C. There is a constant surface current flowing out of the estuary with a midstream velocity of between 6 and 12 inches/sec. The velocity varies in different parts of the estuary and is possibly influenced by the tide and the wind. As the tide falls, the wedge of cold, saline bottom water retracts allowing the other layers to settle at a lower level. Minor differences due to seasonal variations in temperature and runoff will affect conditions during the early and late parts of the season. The overall pattern may remain the same throughout the year, as

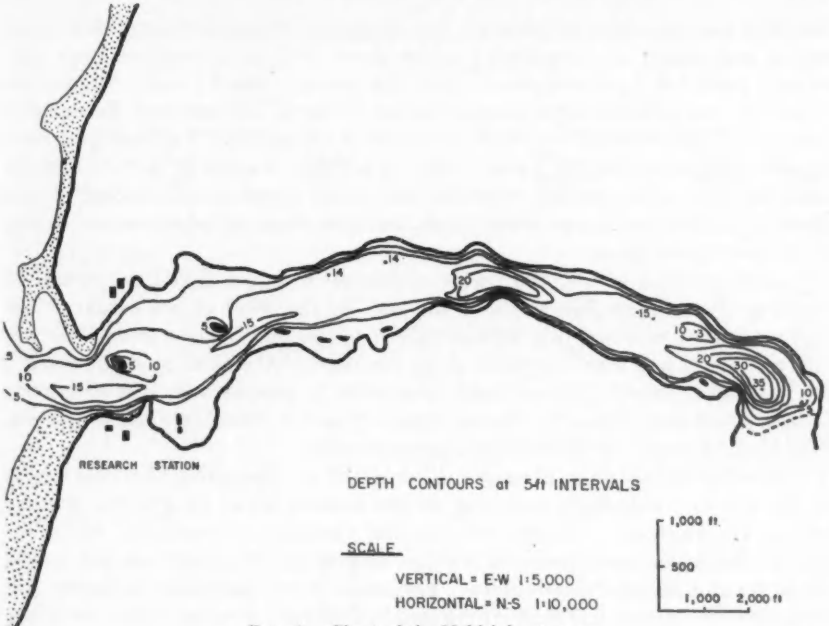

FIG. 1. Chart of the Nabisipi estuary.

salinity differences are probably sufficient to maintain the equilibrium of the water masses during winter, when temperature differences will disappear or even be reversed.

TAGGING

Between May 26 and August 21, 797 juvenile salmon were marked with numbered orange Carlin tags in the estuary of Nabisipi River. The fish were caught in a large 0.75-inch-mesh seine operated from sand banks near the mouth of the estuary and occasionally in other suitable locations. They were carefully transferred to 10-gal plastic tanks and held in these until tagged. The tags were attached by stainless steel wire threaded through the basal cartilages at anterior ends of the dorsal fin. The fork length of each fish was recorded in millimeters. Immediately after tagging the fish were released from the beach in front of the research station, which is on the east bank of the estuary about 100 yards from the sea. During the summer 348 marked fish were recaptured, measured, and re-released. Some fish were handled as many as six times giving a total of 598 recaptures.

COLLECTION AND EXAMINATION OF FISH

An attempt was made to obtain three samples of 100 parr at 14-day intervals from the group of fish that were tagged in the marking experiment.

The first sample, obtained between July 20 and 22, was drawn from that group which could easily be recognized because about 50% of its members bore tags. Most tagged fish had disappeared from the seining areas by early August and were not encountered again during the remainder of the summer. Subsequent samples of parr were very difficult to obtain in the estuary. Fifty-six parr were examined between August 3 and 5, and 75 between August 17 and 21. Almost total absence of tagged fish from the catches at these times, coupled with a decrease in the average size of the catch, indicates these samples were not drawn from the original group.

Two samples of 100 parr were obtained from the lake-like section of Nabisipi River immediately above the falls at the head of the estuary. The first was taken between July 24 and 26 and the second 28 days later. The majority of these fish were captured along the edge of the river at night using a dip net and a lantern. It was found impossible to capture sufficient specimens using a small seine either by day or night. These fish were intended to provide data for comparison with the estuarine population.

Routine examination of each fish consisted of: measuring the fork length to the nearest millimeter, weighing to the nearest tenth of a gram, making note of the stomach contents, the sex, and the state of maturity. Scales for age determination were removed from an area of the back just anterior to the dorsal fin and above the lateral line. The scales were cleaned immediately and smeared onto cellophane folders. Prepared in this way, they are ready for direct examination.

RESULTS

Age, Size, and Condition

Interpretation of scales from the juvenile salmon proved to be difficult. In order to prevent bias in favour of clearer scales, which may belong to faster-growing fish, the age of every fish was estimated from its scale smear. Initially, only about 70% agreement between first and second estimates of age was obtained, but with practice agreement reached between 90 and 95%. With this level of correspondence, some confidence can be placed in the results.

The results (Tables I and II) show that it is best to treat each sample of fish individually. In the estuary, the size of fish in any particular age-group decreased between the first and second samples. Evidence from tagging showed the two groups of fish came from different populations. There was an increase in the size of the 1+ and 3+ age-groups, between the second and third samples, but the 2+ group, which was poorly represented, appeared to decrease in size. It is uncertain whether these samples can be regarded as having been drawn from the same population. In the river, two groups of parr were examined. During the 28-day period between samples, no increase in size can be demonstrated within age-groups and there was, if anything, a decrease in condition. This is difficult to explain unless it is again assumed that the two samples were obtained from different populations, or little or no growth occurred during the interval.

TABLE I. Age, length, weight, and condition of salmon parr from the estuary of Nabisipi River.

	Female					Male				
Age	No.	Mean length (*mm*)	SD	Mean weight (*g*)	Mean K	No.	Mean length (*mm*)	SD	Mean weight (*g*)	Mean K
July 20–22, 1961										
1+	13	87	6.5	7.4	1.12	11	84	7.4	6.9	1.15
2+	8	115	13.3	16.3	1.03	6	120	13.4	18.5	1.03
3+	35	138	16.1	27.8	1.02	20	142	19.2	30.3	1.02
4+	3	139	–	27.5	1.02	4	165	–	50.0	1.00
August 3–5, 1961										
1+	4	78	–	5.1	1.06	6	80	2.6	5.5	1.06
2+	7	100	12.3	10.9	1.03	3	121	–	17.8	1.00
3+	20	126	12.6	21.2	1.03	10	128	15.4	21.9	1.02
4+						6	138	15.3	27.6	1.01
August 17–21, 1961										
1+	15	100	9.7	10.4	1.02	9	94	11.0	8.9	1.07
2+	4	101	–	10.3	0.94	10	101	15.3	11.3	1.02
3+	19	139	11.4	27.6	1.02	15	137	10.5	27.7	1.07
4+	1	120	–	20.0	1.16	2	154	–	29.2	1.08
5+	1	143	–	29.5	1.01					

TABLE II. Age, length, weight, and condition of salmon parr from the lower reaches of Nabisipi River.

	Female					Male				
Age	No.	Mean length (*mm*)	SD	Mean weight (*g*)	Mean K	No.	Mean length (*mm*)	SD	Mean weight (*g*)	Mean K
July 24–26, 1961										
1+	8	73	5.0	4.6	1.19	5	79	2.8	5.6	1.15
2+	9	100	7.7	10.6	1.03	10	104	7.8	11.5	1.02
3+	38	112	6.6	14.1	0.99	27	111	7.6	14.1	1.02
4+	1	123	–	16.5	0.89	2	115	–	16.3	1.06
August 21–22, 1961										
1+	7	80	12.6	5.6	1.04	3	76	–	4.8	1.09
2+	9	101	7.0	9.9	0.95	7	106	13.3	11.6	0.95
3+	45	113	7.6	13.8	0.94	27	114	7.2	14.2	0.94
4+	2	119	–	15.0	0.90	1	109	–	12.5	0.97

When the length of parr taken in the lower reaches of the river is plotted against age, a curve is produced (Fig. 2) which indicates growth rate declined rapidly with increasing age. This is largely an artefact due to the fact that older age-groups consist mainly of slowly growing parr whose more successful siblings have migrated at an earlier age. This is a common phenomenon and has been well documented by Elson (1957). It is not possible to demonstrate the same effect in the estuary because the higher growth rate and heterogeneous background of the parr obscures the patterns.

Mean condition factors (K) for all groups of parr are given in Tables I and II. These have been calculated using the formula:

$$\text{mean K} = \frac{\sum \frac{100W}{L^3}}{N}$$

where W = weight in grams, L = length in centimeters, and N = number in sample.

In the river, condition factors are generally less than 1.0, and in the estuary slightly above 1.0.

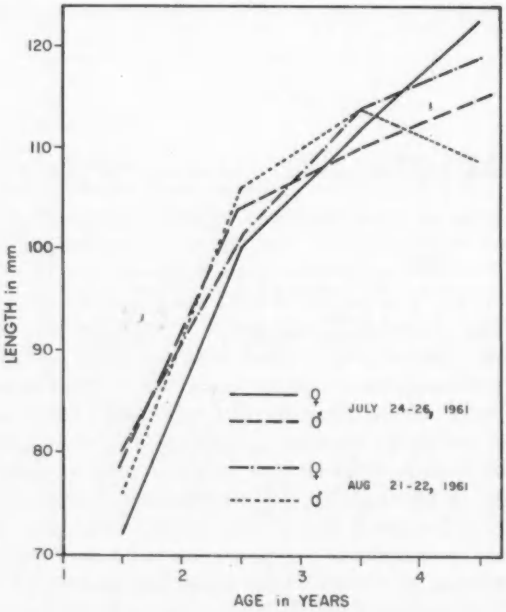

Fig. 2. Age–length relationship of salmon parr in the lower reaches of Nabisipi River.

SEX RATIOS AND MATURITY

All information pertaining to sex ratios can be extracted from Tables I and II. A total of 432 parr were examined of which 249 or 57.6% were females and 183 or 42.4% males. This is broken down into age-groups in Table III.

Males predominate only in the 4+ age-group. Belding (1937) working in Newfoundland and Jones (1949) working in Great Britain, have both found that males tend to predominate in the oldest age-groups of parr.

All the parr examined during this investigation had undeveloped gonads. No males showed even a slight enlargement of the testes.

DIET

Routine examination of each fish included inspection of the contents of the stomach. Recognizable food organisms were noted and recorded with other data on the specimen. From each batch of fish, a representative sample of stomach contents was preserved in 5% formalin. These samples were later analysed in the laboratory to provide a more detailed inventory of the diet. The procedure adopte was as follows: Each sample was placed in a petri dish and floated out in water. It was examined carefully under a binocular microscope. In this way, almost all the food organisms could be identified, and listed so as to indicate the approximate order of abundance. Finally, an estimate was made of the percentage by bulk of each constituent in the sample, thereby giving an indication of the relative importance of each type of food.

TABLE III. Sex ratios of salmon parr in the estuary and lower reaches of Nabisipi River.

	Females		Males	
Age	Number	Percentage	Number	Percentage
1+	47	58	34	42
2+	37	50.7	36	49.3
3+	157	61.6	98	38.4
4+	7	31.8	15	68.2
5+	1			

The results are presented in two ways. Table IV summarizes the data obtained during routine inspection. The numbers opposite each food item indicate the number of stomachs in which this item was recorded. It is at once obvious that the diet of parr living in the estuary is not the same as that of parr in the river. Different food organisms are available to the two groups and this is reflected in the feeding habits. Table V gives the results of analysis

TABLE IV. Frequency of occurrence of food organisms recognized during routine examination of stomachs of salmon parr.

	Estuary			River	
No. of stomachs examined:	100 (July 20–22)	56 (Aug. 3–5)	76 (Aug. 17–21)	84 (July 24–26)	59 (Aug. 21–22)
Unidentified insects	11	22	...	67	41
Calypterate Diptera	15	5	...	2	...
Chironomids	12	2	5	13	...
Corixidae	1	...	...	15	25
Terrestrial Coleoptera	1	...	...	2	...
Ephemeroptera	...	...	...	6	...
Plecoptera	...	...	...	4	...
Homoptera	...	...	...	6	...
Lepidoptera	...	...	...	3	1
Hymenoptera	...	...	...	2	...
Trichoptera	...	...	...	1	2
Amphipoda	9	24	64	...	...
Mysidacea	...	6	1	...	...
Mollusca	...	1	...	...	2
Capelin eggs	57	3	...	...	...
Fish remains	3	3	2	...	...
Sticklebacks	...	...	3	...	...
Unknown	7	...	1	...	...
Empty	11	2	5	...	2

TABLE V. Analysis of the diet of salmon parr showing the percentage by bulk of various items in representative stomach samples.

	Estuary			River	
	July 20–22	Aug. 3–5	Aug. 17–21	July 24–26	Aug. 21–22
Corixidae	...	...	...	31.4	69.6
Chironomid larvae	4.2	7.5	1.5	2.4	0.8
Chironomid pupae	...	9.0	6.0	0.9	0.6
Chironomid imago	0.3	...	...	3.7	...
Calypterate Diptera	16.5	4.5	...	7.9	10.0
Calypterate larvae	6.2	...	...	...	...
Terrestrial Coleoptera	...	...	...	6.4	1.0
Coleoptera larvae	...	0.1	...	...	...
Ephemeroptera nymph	...	...	...	18.6	...
Ephemeroptera imago	...	...	...	...	1.0
Trichoptera larvae	...	0.8	...	3.6	4.0
Trichoptera imago	...	...	...	16.3	...
Lepidoptera	...	...	...	...	5.0
Homoptera	...	0.4	...	7.4	2.0
Plecoptera imago	...	...	...	...	1.0
Hymenoptera	...	0.5	1.0	...	2.0
Simulium larvae	...	0.2	...	...	...
Total insects:	27.2	23.0	8.5	98.6	97.0
Arachnida	...	...	...	1.4	...
Mysidacea	...	31.7	...	...	...
Amphipoda	0.3	40.0	91.5	...	...
Gastropoda	...	1.8	...	...	3.0
Capelin eggs	47.5	0.5	...	...	...
Ammodytes	25.0	3.0	...	...	...
Total others:	72.8	77.0	91.5	1.4	3.0

of stomach samples. A more detailed list of constituents of the diet is presented. The overall picture is the same.

An observation not demonstrated in the tables is that parr in the estuary have a greater quantity of food in their stomachs than those in the river. This is certainly related to a greater abundance of food in this habitat. Amphipods are particularly plentiful and parr in the estuary are often gorged with them. In the river, although almost all stomachs examined contained some food, many contained only a small amount. It should be emphasized, however, that the lake-like section of river immediately above the estuary is not typical salmon habitat. The bottom is mostly muddy with grass growing along the banks, and the current is slight. Corixids, which form the major constituent of the diet of parr in this section of the river, are typically pond-dwelling insects and are not normally available in quantity for salmon to feed on. Stream-dwelling insects are probably more abundantly represented in the diet of parr in other parts of the river.

Growth Rate of Tagged Juvenile Salmon

All tagged fish are referred to as juveniles with no attempt to distinguish between parr pre- or post-smolt stages. All were above 10 cm in length, and most had the appearance of parr or pre-smolts. A few were smolts. Biologically perhaps all of them should be considered smolts. The growth curves of all fish recaptured more than three times (Fig. 3) show a variety of growth rates from zero, over intervals of a few to several days, to a high rate: 19 mm for one fish in 10 days, and 8 mm in 5 days for another. The average rate for all the fish included in this figure is 0.53 mm/day. A number of fish which were handled frequently grew only slowly and some others grew slowly at first but more rapidly later, indicating that tagging and frequent handling has an adverse effect on growth.

Effect of Tagging on Growth

The first few days after tagging, normal behaviour of young salmon is upset. What happens is not known but the chances of recapturing a tagged fish during the first 4 days after release are considerably less than during the subsequent 4 days. In Fig. 4 the interval between tagging and first recapture is shown for 335 of the 348 fish recaptured at least once. Thirteen fish are excluded because of incomplete data. Most fish are recaptured for the first time 6, 7, or 8 days after tagging. It seems that immediately on being released after marking, a fish avoids the normal feeding and swimming activities of the other juvenile salmon, until it has recovered from the operation and adjusted to the presence of the tag. After about 4 days it begins to participate in normal activities again and is vulnerable to recapture. During the period of adjustment the growth rate is reduced. In Fig. 5 the daily growth increment and the total increment are plotted against the interval between tagging and first recapture for the 335 fish in Fig. 4. The points on the graph are means over 3-day intervals. The

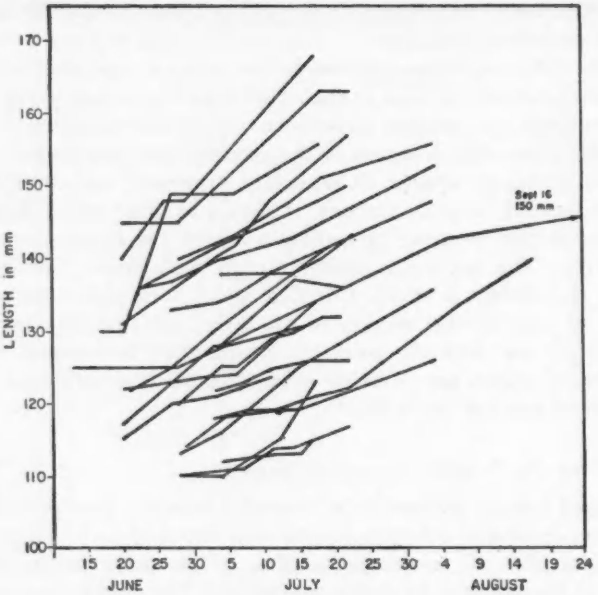

FIG. 3. Growth curves for all tagged juvenile salmon recaptured more than three times (Nabisipi estuary 1961).

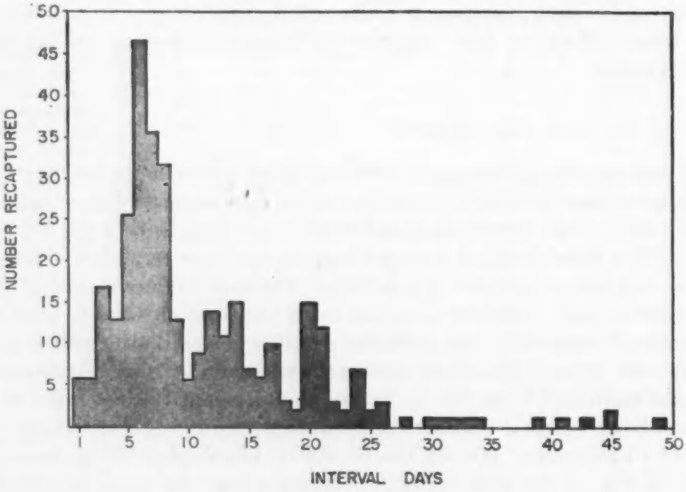

FIG. 4. The interval between tagging and first recapture for juvenile salmon tagged in Nabisipi estuary, June and July 1961.

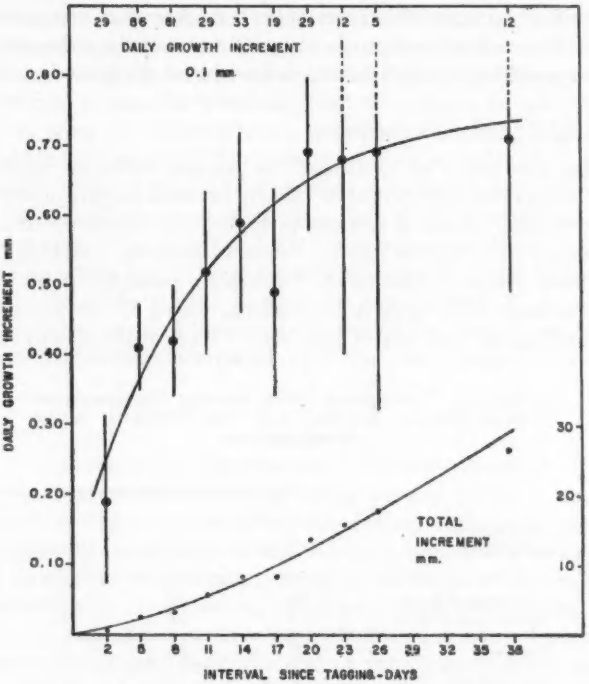

FIG. 5. Daily growth increment, and total growth increment for young salmon tagged in Nabisipi estuary in 1961. The number of measurements for each interval is given along the top of the figure; vertical lines on either side of the points on the curve for daily increment mark the 95% confidence intervals about the means.

curves are drawn freehand. The number of measurements included in each point are given along the top of the graph. Confidence intervals at the 95% level of probability are shown as vertical lines on either side of the means for the daily increment. The confidence intervals are widely spaced because there is a great deal of variation in the growth rates of individual fish and in their reaction to tagging. The confidence intervals, however, confirm that the trends shown in the graph are quite reliable.

Daily growth increments increase as the interval between tagging and first recapture lengthens to about 20 days, when the increment is 0.7 mm/day. This increment presumably approaches the rate of growth of undisturbed fish in the population, which during the period of June and July may grow about 0.8 mm/day. A 120- or 130-mm parr that enters the estuary in early June will grow to a size of 170–180 mm by August and, if it remains in the estuary much longer, may exceed 200 mm in length. In 1960 some fish of this size were caught in the estuary in late August so that a growth rate of 0.8 mm/day seems to agree with observations. This increment is higher than the average of 0.53 mm/day for the multiple recaptures (Fig. 3). Some of the difference is probably the result of handling which, like tagging, reduces the growth rate of the fish.

EFFECT OF HANDLING ON GROWTH

To show the effect of handling, the adverse effect of tagging must be eliminated. By leaving an interval of 18 days between tagging and first handling the number of observations is reduced to 46, but it is hoped the effect of tagging will be removed. In order to test the effect of frequent handling, contingency tables have been prepared which clearly indicate an association between frequent handling and small daily growth increments. Table VI shows the association between handling at intervals of less than 5 days with growth increments of less than 0.4 mm/day and the same association when the interval is extended to less than 10 days. In both cases the value of Chi-squared with one degree of freedom gives a probability of less than .005 and it must be concluded that frequent handling is associated with small growth increments. While it is not possible to express the effect of handling in reliable numerical values, an indication of the effect can be obtained from the following figures. Thirty fish handled at intervals of less than 10 days (mean interval 4.1 days) had a mean daily increment of 0.29 mm and 16 fish handled at intervals of 10 or more days (mean interval 20.25) had a mean daily increment of 0.52 mm.

TABLE VI. Contingency tables showing the association between frequent handling and poor growth of tagged juvenile salmon.

Handling at intervals of	Daily growth increment (*mm*)		
	<0.4	>0.4	Totals
Less than 5 days	16	5	21
More than 5 days	7	18	25
Totals:	23	23	46
$\chi^2 = 10.6$ P is less than .005			
Less than 10 days	23	7	30
More than 10 days	3	13	16
Totals:	26	20	46
$\chi^2 = 14.2$ P is less than .005			

ESTIMATE OF SIZE OF POPULATION

One of the aims of the tagging experiment was to determine the number of juvenile salmon over 100 mm in length resident in the estuary of Nabisipi

River. For a number of reasons this could not be done except in a very general way. The major sources of potential error were: the possibility that the group of salmon in the estuary was not stable, that tagged fish behaved differently than untagged fish, at least for a few days following marking, and the fact that returns from tagging on different days varied considerably. Eight estimates of the population, using results from days when it was felt that over 30 fish had been successfully tagged and adequate recaptures accumulated, range from 741 to 2525. Combined results for these days gives an estimated population of slightly over 1000. This is felt to be a fair estimate of the number of young salmon over 100 mm in length resident near the mouth of the estuary of Nabisipi River in June and July 1961. There were more salmon in the estuary than this. Seining in other parts of the estuary produced a few salmon parr, but the numbers were insignificant compared with the concentration found near the mouth.

DISCUSSION

Before deciding whether stocks of young salmon living in the estuary of Nabisipi River can influence the salmon-producing capacity of the river it is worth considering the origin of these fish. Hydrographic and physical conditions preclude the possibility of salmon spawning in the estuary. Physical conditions make it probable that no spawning occurs in the lake-like section of river immediately above the estuary. It is unlikely that any spawning occurs in the falls separating the estuary from the rest of the river. It must be concluded that most of the young salmon in the lower reaches of the river and estuary have migrated there from spawning areas farther up stream. The predominance of 3-year-old fish in these parts of the river suggests that this is the most mobile age-group. Many are fish which, judging by their size, just failed to become smolts in the spring or are on the point of becoming smolts. Scales of 844 adult salmon taken in the estuary of Nabisipi River in the years 1960–64 show that 32% of them migrated after 3 years in the river and 56% after 4 years. In view of this, it seems probable that the majority of the 3-year old parr in the river spend another winter in fresh water before migrating, whereas it is likely that those already in the estuary migrate the same year.

It appears probable that two kinds of young salmon were encountered in the estuary in 1961. The stock that was tagged, and from which the sample of July 22–23 was drawn, is part of the spring run of migrants from the river which have remained in the estuary because of favourable conditions or because they had not completed metamorphosis. In addition, there is a small resident stock of salmon parr in the estuary which has moved or been accidentally washed into the estuary from the river. Parr of this type populating the estuary of the Margaree River were described by Huntsman (1945). He quotes unpublished data by P. F. Elson which indicate freshets are important in stimulating downstream movement but that some downstream movement of parr occurs in the absence of this stimulus.

The number of juvenile salmon in the estuary of Nabisipi River changes considerably from year to year. In 1960, the sand spit across the western part of the estuary was well developed and a large population of young salmon was present in the estuary into late August. The summer of 1960 followed severe flooding in the river. In 1961, the number of young salmon in the estuary was considerably reduced and the number diminished still more in 1962. It is possible that 1960 was an unusual situation resulting from the floods. Alternatively, spring migrants may remain much longer in the estuary in certain years, perhaps being influenced by the degree of impoundment provided by the sand spit. This could result in the situations observed during the years 1960–62.

It is concluded that, although the estuary provides a suitable habitat for young salmon, it is certain the number maturing there is insignificant compared with the number in the rest of the river. The estuary may be a temporary haven for partly metamorphosed smolts during the early summer, until they are physiologically ready to move off into the sea. There appears to be an ample supply of food and young salmon grow well in this habitat. They do not, however, grow well enough to excite the possibility of deliberately introducing young salmon into the estuary, or trying to delay the departure of young salmon arriving there normally. In contrast to the estuary it seems that the lower reaches of Nabisipi River are not productive salmon water. The stock of young salmon is low, the stomachs of parr living there contain little food, and their condition factors appear low.

The tagging experiment designed to provide information on the numbers of young salmon in the estuary and on movements of Nabisipi fish produced some interesting data on the effects of tagging on behaviour and growth of young salmon. As far as is known this is the first time this has been demonstrated in a salmon-marking experiment in the field. Mills (1958), on the basis of a few results obtained in a hatchery, concluded that tagging had little effect on the growth of salmon parr. Our results show that, while tagging can give a general indication of the growth rate of wild fish, the rate will always be low compared with untagged fish, particularly if there is frequent handling. For intervals of time in excess of 4 weeks the rate approaches that of unmolested fish. Only when the interval is long will the effect of tagging become negligible.

ACKNOWLEDGMENTS

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Temperature of Maximum Density of Fresh Water and its Effect on Circulation in Great Bear Lake[1]

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ABSTRACT

Theoretical aspects of the influence of the temperature of maximum density of water on the circulation of deep cold lakes are applied to the temperature structure of Great Bear Lake. In general good agreement between observation and theory is found. In most years summer circulation does not extend below a depth of 225 m as stability at lower levels is sufficient to prevent it. In cold years with slow heating, circulation extends to the bottom (450 m). In winter the lower half of the lake is isothermal at the temperature of maximum stability (3.53 C) of the bottom water. This is explained on the basis of the slope of the line of maximum density being double that of the slope of the line of maximum stability. When the water at a depth of 225 m is cooled to 3.53 C the point of maximum temperature in the profile at this depth is removed so that isothermy develops in the lower half of the lake.

INTRODUCTION

Circulation in a body of water is dependent upon the interaction of the internal forces leading towards stability and the external forces producing turbulence. In fresh water stability results from the temperature structure, although in certain waters turbidity and dissolved salts can modify the situation. The external forces are, to a large extent, the result of shearing stresses set up by the wind blowing across the lake surface. When the turbulence thus produced is sufficient to overcome the stability, circulation takes place; this may occur in either the whole lake or that part of it where the stability is inadequate to counteract the degree of turbulence encountered.

According to the classical interpretation, the spring heating of a lake, the surface temperature of which has fallen below the temperature of maximum density of water, causes a rise in temperature of the surface water; this water sinks towards the bottom until the whole lake reaches a uniform condition. When these convection currents are reinforced by small external forces complete overturn results. Continued heating causes a more stable structure to develop so that circulation is restricted to the surface layers; this is followed in the fall by a second period of instability as the surface waters cool to 4 C. If the lake is sufficiently deep for cooling to be restricted to the upper layers the bottom waters will remain close to 4 C throughout the winter.

Observations on deep, cold lakes showed that, contrary to theory, there is a gradual decrease in temperature below 4 C with increasing depth. Strøm

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(1945) constructed an envelope from known temperature profiles of deep lakes to show that a straight line could be drawn separating those with surface temperatures above and below 4 C. This line he interpreted as representing the change of temperature of maximum density with depth. Eklund (1963) challenged this view on the basis of values he obtained from the solution of an equation of state derived from compressibility data computed from sonic velocities. The value for the rate of decrease of temperature of maximum density with pressure was exactly double that of Strøm. Johnson (1964) was doubtful of the new values as they appeared to provide no explanation for the line deduced by Strøm which seemed to have a good physical basis. Subsequently, Eklund, in a very elegant analysis, derived an equation which provides a measure of the stability ($\emptyset$) of a lake at any given depth:

$$\emptyset = v_0 \, (-bp + 2c\Theta) \, (rb/2c)$$

where v_0 is the specific volume of water at the temperature of maximum density and atmospheric pressure, p is the "gauge" pressure, b is a constant derived from the adiabatic and isothermal compressibility of water and c is the rate of change of specific volume with temperature between 3 and 4 C. Eklund showed that when $r = \frac{1}{2}$ the value of $\emptyset$ is minimal and the stability is greatest, when $r = 1$ the profile is at maximum density throughout and stability is zero and when $r = 0$ the profile is isothermal and again stability is absent. This means that maximum stability is achieved when the decrease in temperature with pressure is half the decrease in temperature of maximum density. This line follows very closely the line derived by Strøm and lies midway between the line of maximum density and the isothermal profile at 4 C. A corollary to this formulation showed that if the water column is stable the temperature–depth curve must change its sign of slope where it crosses the line of maximum density.

Observations pertinent to the above discussion were made on Great Bear Lake in the Canadian Northwest Territories over the period 1963–65 and include both winter and summer recordings.

Great Bear Lake (Fig. 1) lies between latitudes 64°40′ and 67°0′N; it has a total area of 31,153 km (12,029 miles), a maximum depth of 452 m (1485 ft) and a mean depth of 76 m (250 ft). The lake is composed of five arms joined by a central region. Four of the arms have a maximum depth of approximately 120 m (400 ft) whilst McTavish Arm, with a mean depth of 107 m (351 ft), contains the deepest water in an area 6 miles west of Port Radium.

In this area of deep water, at the time of maximum heat intake in the second half of August, surface temperatures of 5–7 C are normal, but in cold summers the surface may not reach 4 C. The lake is extermely oligotrophic; secchi disc readings of between 20 and 30 m have been obtained and on rather limited photometer readings the vertical extinction coefficient appears to be about 0.14. The total dissolved solid content is low with a value of 79 ppm.

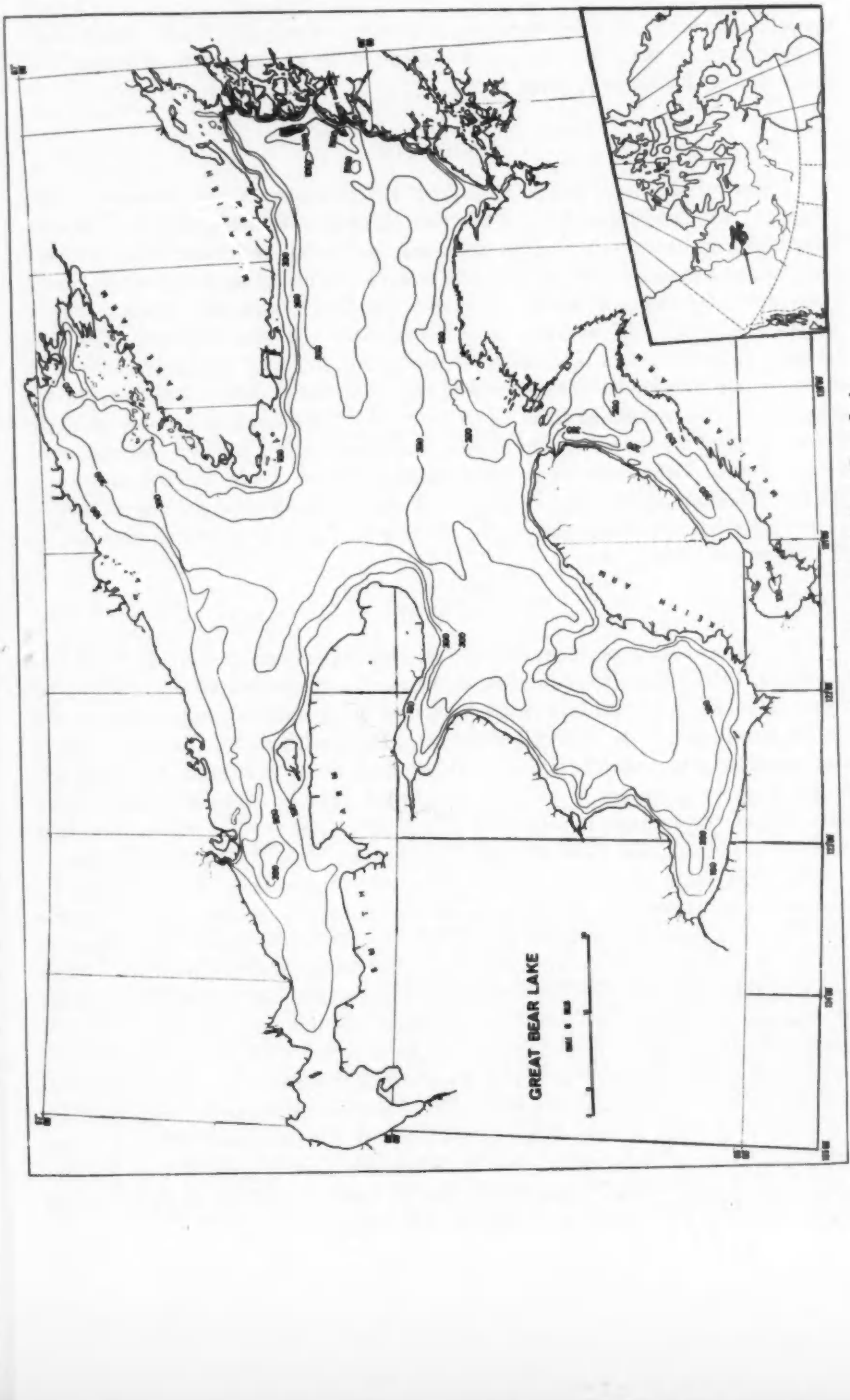
GREAT BEAR LAKE
SMITH ARM
KEITH ARM

FIG. 1. Bathymetric map of Great Bear Lake.

Ice-cover develops in November and reaches a maximum thickness of 2 m in April; break-up begins in late June or early July and over the period of study the lake became ice-free between July 5 (1963) and July 23 (1965). Observations on temperature structure are not complicated by the presence of glacial melt water or the discharge of large rivers.

METHODS

Observations were made from the 115-ft motor vessel *Radium Gilbert* (Capt A. McInnes) chartered from the Northern Transportation Company. Temperatures were taken with deep-sea, protected reversing thermometers read to the nearest 1/100 degree; the standard error of such an instrument is close to ±.02 degrees when corrected for depth and the temperature at which it is read. All readings were made after the thermometers had been allowed to stabilize at laboratory temperature. Oxygen determinations were made by the modified Winkler method following Strickland (1960); percentage saturation was computed from Mortimer (1956) although it appears that the values he used for the solubility of oxygen were up to 3% too low (Montgomery et al., 1964). Other chemical determinations were made by the Industrial Waters Division of the Department of Mines and Technical Surveys, Ottawa, after removing all solids above the diameter of .45μ by passing through an HA Millipore filter.

RESULTS

The temperature profiles obtained in 1963 have been previously published (Johnson, 1964); these were in close agreement with the material published by Strøm, the late July and late August profiles being narrowly separated by the line he concluded to be that of maximum density with depth. Eklund's line of maximum density cuts the July profile at a point of maximum temperature. From these two profiles it may be concluded that no circulation has taken place during the summer months at a depth below 200 m since the temperature structure has remained close to that of maximum stability at these depths.

In 1964 more extensive observations were made (Fig. 2 and Table I). In each case, where the temperature at the surface is below 4 C, the profile cuts Eklund's line of maximum density at a point where there is a change of sign of the slope. This point occurs at the maximum temperature of the profile if the immediate effects of surface heating are discounted. The profile for August 27 comes very close to following the line of maximum stability below a depth of 100 m. If isotherms for the seasonal changes of temperature are plotted against depth (Fig. 3) it is apparent that there is no circulation below a depth of 225 m. Measurement of oxygen content indicates a slight decline in the percentage saturation value as the bottom is approached. When account is taken of the error involved in the data Mortimer used it appears that bottom values are slightly below saturation. Nitrate and phosphate determinations (Table II) show no discernible variation with depth.

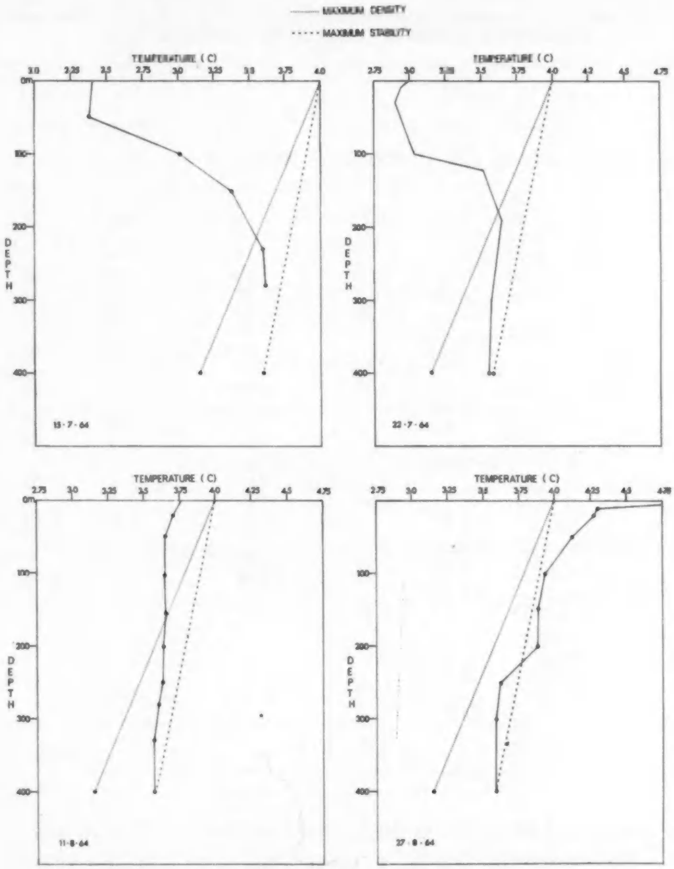

FIG. 2. Temperature profiles at the station 6 miles west of Port Radium for the year 1964.

In April 1965, observations were made through the ice at a time when lake temperatures might be expected to be minimal. Above 150 m the profile (Fig. 4) follows a sigmoid curve with a rapid rise in temperature at a depth of 50 m. Below 200 m the unexpected result of isothermy at 3.52 C was obtained. Oxygen, nitrate, silica, and phosphate show a similar pattern to that previously observed. The anomalous figure for silica at a depth of 100 m is most likely an error as none of the other data supports such an abrupt change.

After a late break-up the summer of 1965 turned cold and windy. By August 2 surface temperatures in the area of deep water had reached only 2.75 C, but below 200 m there was a very slight tendency towards stability. By the 23rd of the month when heat intake is close to maximum the surface

TABLE I. The recorded temperatures at all depths obtained in the years 1964 and 1965 at a station 6 miles west of Port Radium, Great Bear Lake.

	1964					1965		
Depth (*m*)	July 15	July 22	Aug. 11	Aug. 27	Depth (*m*)	Apr. 9	Aug. 2	Aug. 25
0	2.36	3.00	3.77	5.22	0	ice	2.75	3.54
3		2.92			2	.04		
5		2.92			5	.04		
10		2.93		4.30	10	.04	2.68	
19			3.71		20	.06	2.64	
20				4.26	30	.18		
30		2.90			49			3.42
48			3.65		50	1.37	2.68	
50	2.33			4.12	99			3.41
100	3.02	3.03		3.93	100	2.91	2.66	
106			3.64		148			3.43
123		3.52			150	3.50	2.67	
150	3.37	3.57		3.87	200	3.53	3.55	
155			3.65		246			3.42
188		3.64			250	3.53	3.55	
200		3.63		3.87	295			3.43
201			3.63		300	3.51	3.53	
230	3.59				350	3.52	3.55	
250			3.62	3.62	390	3.52		
280	3.61		3.60		400		3.51	
299			3.59					
300		3.57		3.59				
317		3.57						
328			3.57					
347			3.57					
350				3.59				
396			3.56					
398		3.56						
400		3.56		3.58				

had only attained 3.54 C; below 50 m there occurred an isothermal condition at 3.42 C. Unfortunately, due to an error discovered later the readings from thermometers at the lower levels proved unacceptable.

DISCUSSION

The above results show that circulation in Great Bear Lake is irregular and may take place at different times of the year above and below 225 m.

Circulation in the lower 225 m appears to be governed by the relationship of actual temperature to the lines of maximum density and maximum stability as deduced by Eklund. In most years sufficient stability develops early in the season to prevent circulation taking place in the lower part of the system, the structure being maintained close to that of maximum stability. In cold years such as 1965 the slight degree of stability established is inadequate to prevent complete circulation at the height of the summer.

In the years observed the profiles in the early part of the season all cross the line of maximum density at the point of maximum temperature; this is in

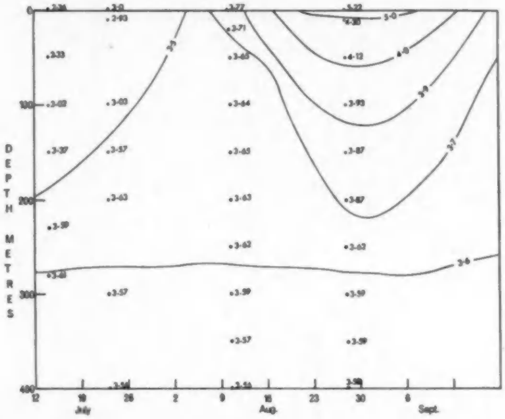

Fig. 3. Isotherms for 1964 at the station 6 miles west of Port Radium.

Table II. The concentration of oxygen, nitrate, silica, and phosphate as determined on August 27, 1964, and April 9, 1965.

Depth	Oxygen (*mg/liter*)	Oxygen (*% sat*)	Nitrate (NO₃) (*ppm*)	Silica (SiO₂) (*ppm*)	Total phosphate (PO₄) (*ppm*)
August 27, 1964					
0	13.21	109	.58		< .01
10	14.00	112			
20	13.95	112			
50	13.79	110	.58		.01
100	13.40	108	.43		.02
150	13.71	109			
200	13.73	109	.49		< .01
250	13.20	105			
300	13.48	106	.55		.02
350	13.05	103	.46		< .01
400	12.78	102			
April 9, 1965					
2	14.40	104	.9	2.4	< .01
10	14.37	104	1.0	2.4	< .01
20			.9	2.4	< .01
30	14.06	101			
50			.9	2.4	< .01
100	12.87	100	.9	6.8	< .01
150			.9	2.2	< .01
200	12.62	100	1.0	2.0	< .01
300	12.56	100	.9	2.0	< .01
350	12.53	100	.9	2.2	< .01
390	12.60	100	1.0	2.4	< .01

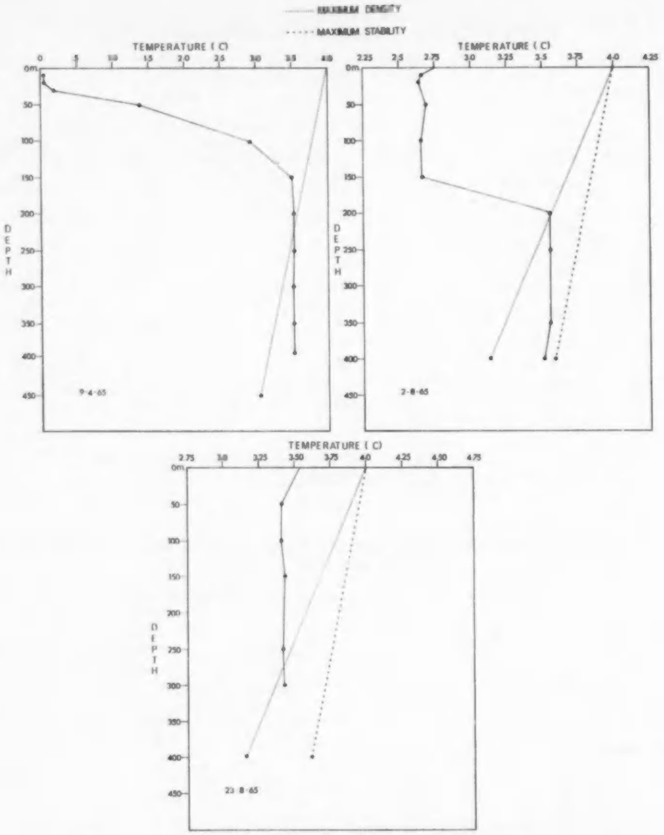

FIG. 4. Temperature profiles at the station 6 miles west of Port Radium for the year 1965.

conformity with Eklund's thesis. It should also be noted that this is the highest temperature of the profile if immediate surface heating is discounted. Above this point cold water lies above warm and below it warm water lies on top of cold.

The winter condition of isothermy below 200 m can be satisfactorily explained on the basis of Eklund's computations. If the lake enters the winter cooling period with stability below a depth of 200 m, the bottom water will be close to 3.53 C which is the temperature of maximum stability for the greatest depth (450 m). As cooling of the surface waters continues the profile will move to the left (Fig. 5) with a gradual lowering of the temperature at which it crosses the line of maximum density. If the upper layer loses sufficient heat a point will eventually be reached when the profile cuts the line of maximum

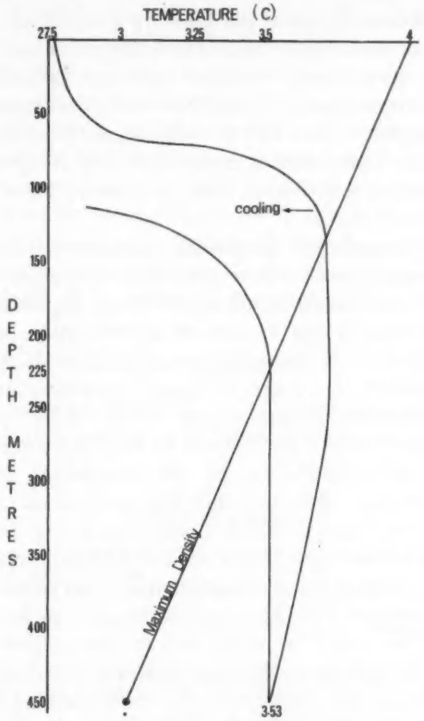

FIG. 5. Diagram of the mechanism operating to produce isothermy in the deep water during winter. The profile moves from right to left as cooling proceeds, until it reaches the point where it cuts the line of maximum density at a temperature of 3.53 C. When this occurs the profile becomes isothermal below 225 m.

density at a temperature of 3.53 C. This will occur at a depth of 225 m. When this condition is achieved there will be no point of maximum temperature and the lower half of the lake will be isothermal at 3.53 C. This very closely approaches the condition found on April 9, 1965.

The extent of circulation taking place in this bottom mass of isothermal water is doubtful as the forces normally causing circulation will be minimal. Heating from the centre of the earth will only supply about 6 calories/cm^2 each month (Hart and Steinhart, 1965), which is unlikely to be sufficient to stimulate appreciable convection currents.

Thus, in deep cold lakes there appears to be a division between the water lying above and below the depth at which the temperature of maximum density equals the temperature of maximum stability at the bottom. As the slope of the line of maximum stability is half that of maximum density, this critical plane will occur at the median depth of the lake. In Great Bear Lake this occurs at a depth of 225 m and the influence of this dividing plane can readily be discerned in Fig. 2. In the bottom waters stability is maintained through the summer, whereas the upper layers circulated at about 3.6 C in this particular year (1964).

In 1965 the temperature at 225 m only exceeded 3.53 by .02 C degrees on August 2. Stability is therefore extremely low and it appears that, following the high winds experienced shortly after this date, the whole lake circulated at a mean temperature of 3.42 C.

In spite of this irregular circulation it is apparent from the oxygen values, which do not fall appreciably below complete saturation even at the end of winter, that oxygen depletion does not occur at any depth. Complete circulation was observed only once in the 3 years of investigation, and unless it occurs more regularly in the fall this is sufficient to maintain high oxygen values and the uniform distribution of solutes. If summer stability in deep water is well established fall circulation would appear to be unlikely, but data for this period is not readily obtained on account of weather conditions.

The effect of deep circulation on the production of phytoplankton is likely to be considerable and is probably the main cause of the extreme oligotrophy of the lake. Sverdrup (1952) has related primary production to circulation and light penetration through the use of the term "critical depth," which is that depth above which total photosynthesis is equivalent to total respiration per unit area of surface. For an increase in phytoplankton production to take place circulation must be restricted to the region above the critical depth. Marshall (1958) gives a simplified form of Sverdrup's equation for the determination of the critical depth and the compensation depth which is that depth below which respiration exceeds photosynthesis:

$$D_{cr} \simeq \frac{1}{k}\,\frac{\bar{I}_e}{I_c}$$

$$D_c = \frac{1}{k}\log_e\frac{\bar{I}_e}{I_c}$$

where D_{cr} and D_c are the critical depth and compensation depth, k is the vertical extinction coefficient and $\bar{I}_e$ and I_c are the "effective" energy and the energy reaching the compensation depth. The energy reaching the earth's surface in latitude 60° on August 15 is given by Hutchinson (1957) as approximately 477 ly/day. According to Sverdrup approximately 10% of this will be reflected and of the remainder only 20% will be effective in photosynthesis. Strickland (1958) gives a value of 4.3 ly/day for the energy at the compensation depth in the ocean, and this may be taken as an approximate value for fresh water. Combining these values with the figure of 0.14 for the extinction coefficient given previously, a critical depth of 142 m and a compensation depth of 21.4 m are obtained.

Thus, a depth of circulation of 225 m is well below the critical depth of 142 m for the month of August; at this time, although the lake is warming, phytoplankton production will be extremely low. On the other hand, under the ice in April there is considerable stability in the upper 50 m and it would appear likely that conditions at this time are more favourable for primary production, in spite of the high percentage of light absorbed by the ice. A very similar conclusion was reached by Hobbie (1964) as a result of carbon-14 determinations made on an Alaskan Lake.

Although I have previously expressed objections to Eklund's thesis (Johnson, 1964) it is now apparent that the theory in its present form provides a very good explanation of the observed data from Great Bear Lake.

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Effect of Starvation and Refeeding on Some Liver and Blood Constituents of Atlantic Cod (*Gadus morhua* L.)[1]

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ABSTRACT

Changes in body weight, liver weight, liver glycogen, blood plasma *p*H, hematocrit, plasma protein, and glucose levels in the Atlantic cod (*Gadus morhua* L.) during feeding, fasting, and liver regeneration are recorded. Resting liver glycogen content (37 ± 7 mg/100 g liver) is much lower than in many terrestrial and freshwater vertebrates. There is a transient increase in liver glycogen (702 ± 188 mg/100 g liver) with resumed feeding after prolonged fasting. Average resting levels of plasma protein (4.66 ± 0.36% w/v), glucose (100 ± 4 mg/100 ml plasma) and hematocrit (23.2 ± 1.2% v/v) decreased during the initial stages of food depletion. A reversal of this trend resulting in apparent increased levels is observed in acute malnutrition. This is attributed to dehydration of plasma with consequent drop in the total blood volume. The steady increase in plasma *p*H (7.24–7.54) with the duration of fasting may be due to extensive deamination of tissue protein amino acids for gluconeogenesis.

INTRODUCTION

THE MANIFOLD FUNCTIONS of the mammalian liver have received a great deal of attention. Synthesis of plasma proteins and glycogen from dietary constituents, gluconeogenesis and glycogenolysis, all of which contribute to homeostasis, take place in the liver. Under conditions of stress, e.g. starvation, the mammalian liver can mobilize the breakdown of stored glycogen in order to maintain the blood glucose level within normal limits. The Atlantic cod (*Gadus morhua* L.) is one of many marine vertebrate species which do not have sufficient liver glycogen to sustain the animal when the food supply is restricted. Yet the cod survives several weeks of starvation by using up depot fats to maintain an adequate metabolic pool of carbon substrates. Love (1958) has shown that liver weight and liver fat were reduced during starvation in North Sea cod, the lower limit below which death occurred being about 2% liver fat. A good nutritional state of cod at the time of capture is important as it is reflected in the yield of liver oil, and though indirectly, quality of the fillets.

The nature of the carbon pool (metabolites with 2–6 carbon atoms) and the mechanisms by which neutral lipids are utilized for the production of short-chain carbon substrates by poikilothermic vertebrates have been scantily explored in the past. Studying the changes in glycogen, pyruvate, and lactate during and following muscular activity in rainbow trout (*Salmo gairdneri*), Black et al. (1960, 1962) concluded that fish carbohydrate metabolism under

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conditions of exercise, recovery, and starvation are essentially in accord with the mammalian pattern. MacLeod et al. (1963) demonstrated the presence of many enzymes of the Embden-Meyerhof glycolytic pathway in steelhead trout (*Salmo gairdneri gairdneri*), using tissue extracts and homogenates from heart muscle, skeletal muscle, liver, and kidney. Jonas and Bilinski (1965) have studied the changes in ketone bodies in the blood of salmonoid fishes and observed no appreciable change in acetoacetate and β-hydroxybutyrate levels by starvation.

The present study is concerned with the behaviour of cod liver and blood during starvation and resumed feeding. Changes in body weight, liver weight, liver glycogen, plasma glucose, *p*H, plasma proteins, and hematocrit (packed erythrocyte volume per cent) were recorded after varying intervals of starvation and during active regeneration of the liver when feeding was resumed.

METHODS OF HANDLING

The cod were caught in March and held in filtered, aerated recirculating sea water at 6.5 ± 0.5 C. The fish were acclimated in the aquarium for 7 days and fed chopped herring (*Clupea harengus*) *ad libitum* three times a week for 3 weeks. Feeding was interrupted for 5 weeks and resumed three times a week for 2 weeks for the studies on the regeneration of liver.

The cod were transferred to aerated seawater tanks (ca. 4 C) in the evening and held overnight in the dark. In order to avoid overcrowding, the fish were allowed a minimum per capita volume of 2.5 liters in the tank. They were anaesthetized by adding tricaine methanesulphonate (M.S. 222) to sea water to a final concentration of 40 mg/liter. It took about 20 min for the cod to become immobile. From this point, they were handled as quickly as possible for weighing, tagging, and bleeding, so that no fish remained in the anaesthetic longer than 40 min. They were revived in aerated, flowing sea water and returned to the holding tank until required again. About 2 ml blood were withdrawn from the branchial artery with a sterile, heparinized disposable syringe. Blood samples were transferred immediately to centrifuge tubes in an ice bath and the plasma was separated by centrifugation in the cold room. All samples were stored at 3 C until required for analysis. Livers were frozen in small, leak-proof plastic bags immediately after excising by plunging the bags into a dry ice–alcohol bath. The frozen samples were stored at −27 C until used for glycogen determination. All analyses were concluded usually within 4 days after collection of the samples.

METHODS OF ANALYSES

Plasma glucose was determined by the glucose oxidase micromethod using regular glucostat reagents (Worthington Biochemical Corporation, Freehold, N.J.). The microbiuret method of Itzhaki and Gill (1964) was employed for plasma proteins. Glycogen was determined by the anthrone method according to van der Vies (1954) with one modification: the glycogen was precipitated overnight from trichloroacetic acid extracts by five volumes of ethyl alcohol and the precipitate dissolved in a suitable volume of water for the analysis. Reproducible recoveries of over 98% were obtained for standard glycogen (Fisher Reagent). Plasma *p*H was determined with the Astrup glass microelectrode for blood against a reference calomel electrode using the Radiometer Model 4 *p*H meter at 38 C. Hematocrits were obtained by a microcapillary method.

EXPERIMENTAL

Twenty-seven cod were investigated in the feeding, fasting, and resumed feeding phases. The feeding phase was studied in two stages, arbitrarily designated as "feeding" and "satiated," involving two and eight feedings, respectively, on a three times a week schedule. The fasting phase was subdivided into two stages, depletion I and depletion II, 16 and 30 days after the last feeding. The regeneration phase was studied 2 weeks and six feedings after depletion II.

All the methods were standardized using cod not included in the study. Optimum conditions were determined and the variations introduced by cold storage, and interference by known and unidentified components in the samples were minimized in the final procedures. The preferred order in which the determinations may be undertaken by one analyst appears to be (1) hematocrit, (2) plasma *p*H, (3) plasma proteins, (4) liver glycogen, and (5) plasma glucose.

In each stage of the study at least five cod were killed at random and their livers excised and frozen as described above. Blood samples were taken from all survivors at the start of every series. Thus, in addition to average values for the group, it was also possible to prepare chronological records on many individual cod for a more direct evaluation of behaviour under stress.

RESULTS

Average values for body weight, liver weight, and liver glycogen (Table I), hematocrit, plasma *p*H, proteins, and glucose (Table II) are presented. Starvation resulted in loss of body weight, liver weight, and liver glycogen. The most rapid decrease was observed in liver weight; when expressed as per cent body weight, the reduction was over 50% in 4 weeks. The losses were made up within the 2 weeks when feeding was resumed. Liver glycogen increased several-fold temporarily during regeneration of the liver, although the normal level in satiated cod is only of the order of 40 mg/100 g liver.

There was a slight increase in hematocrit in depletion II. Plasma *p*H increased from 7.24 to 7.54 during 4 weeks of starvation, but rapidly returned to normal in the regeneration phase. Plasma protein and glucose decreased during the fasting phase, although the trend was reversed slightly in acute malnutrition. Both components showed transient increases above satiated levels immediately after feeding was resumed.

The free polysaccharide content of plasma will have some meaning in elucidating the mechanism of dissipation of the transient glycogen during liver regeneration. It is defined in this context as the alcohol-precipitable fraction

TABLE I. Average values[a] for body weight, liver weight, and liver glycogen in cod during fasting, feeding, and liver regeneration.

No. of days from start	Physiological condition	Body weight (*kg*) Mean ± SE	Liver weight (*g*) Mean ± SE	Liver weight (*% body wt*) Mean ± SE	Liver glycogen (*mg/100 g*) Mean ± SE
7	Feeding	1.35 ± 0.07 (27)	–	–	–
21	Satiated	–	37 ± 8 (5)	3.48 ± 0.67 (5)	37 ± 7 (5)
37	Depletion I	1.23 ± 0.06 (21)	45 ± 7 (6)	3.33 ± 0.50 (6)	10 ± 2 (6)
51	Depletion II	1.16 ± 0.06 (14)	14 ± 3 (5)	1.50 ± 0.38 (5)	20 ± 9 (5)
71	Regeneration	1.53 ± 0.07 (7)	58 ± 5 (7)	3.79 ± 0.28 (7)	702 ± 188 (7)

[a] Number of measurements in parentheses.

from deproteinized plasma. The determinations were made on aliquots of pooled plasma after deproteinizing by two treatments with 5% trichloroacetic acid. The polysaccharide fraction not bound to protein was precipitated from the supernatant by 5 volumes of ethyl alcohol and estimated by anthrone reagent. Average values for cod plasma polysaccharides not bound to protein, during feeding, fasting, and liver regeneration, are expressed as glycogen equivalents in Table III. No consistent differences could be detected in the free polysaccharide content of plasma in the four nutritional states studied.

In Table IV are given typical chronological records of one male and one female cod, both of which were killed in the final stage of the experiment.

DISCUSSION

The liver glycogen content of well-fed cod is lower than in most terrestrial and freshwater vertebrates for which data are available. It is reduced further during prolonged fasting. The values for normal liver glycogen (40 mg/100 g) were lower than the lowest value for muscle glycogen of trap cod (203 ± 62 mg/100 g) reported by Fraser et al. (1965). It is therefore likely that liver glycogen plays little, if any, role in the recovery of exhausted muscle. The Cori cycle, operative in many common terrestrial vertebrates, may have to be modified in the case of species with a low glycogen storage potential. In cod, blood lactate resulting from glycolysis in the muscle may be converted to glucose in the liver and released to the blood stream without the mediation of a glycogen bank in the liver. This scheme would be in accord with the observations of Black et al. (1960) that blood lactate removal in rainbow trout following muscular activity is slower than in mammals. However, the lower body temperature and rate of blood circulation are also important factors contributing to the slow disappearance of blood lactate following exercise in fishes.

TABLE II. Average values[a] for Hematocrit (per cent packed erythrocyte volume), plasma pH, plasma protein, and plasma glucose in cod during feeding, fasting, and liver regeneration.

No. of days from start	Physiological condition	Hematocrit		Plasma pH		Plasma protein		Plasma glucose	
		(% v/v) Mean ± SE	t–test p^b	Mean ± SE	t–test p	(% w/v) Mean ± SE	t–test p	(*mg/100 ml*) Mean ± SE	t–test p
7	Feeding	–	–	–	–	5.42 ± 0.33(27)	–	108 ± 8(27)	–
21	Satiated	23.2 ± 1.2(26)	–	7.24 ± .01(2)	–	4.66 ± 0.36(26)	–	100 ± 4(26)	–
37	Depletion I	25.1 ± 1.4(15)	> .05	7.40 ± .03(20)	< .01	4.73 ± 0.40(21)	> .05	72 ± 4(21)	< .01
51	Depletion II	27.0 ± 1.3(14)	< .05	7.54 ± .02(14)	< .01	4.76 ± 0.54(14)	> .05	75 ± 4(14)	< .01
71	Regeneration	26.1 ± 1.5(7)	> .05	7.24 ± .02(7)	1.00	8.57 ± 0.26(7)	< .01	130 ± 6(7)	< .01

[a] Number of measurements in parentheses.

[b] p is the probability of the deviation from satiated mean value being due to sampling variability alone.

TABLE III. Average content of free polysaccharides in deproteinized cod plasma during fasting, feeding, and liver regeneration.

No. of days from start	Physiological condition	Free polysaccharides in plasma glycogen equivalent mg/100 ml
21	Satiated	6.3
37	Depletion I	6.8
51	Depletion II	5.2
71	Regeneration	4.2

The transient but considerable increase in cod liver glycogen (Table I) during active liver regeneration is very likely derived from dietary carbohydrates. While a large proportion of ingested fats are deposited mainly unchanged (Lovern, 1935), exogenous carbohydrates supply the ready source of energy to cope with the increased metabolic activity of the regenerating tissues. On the other hand, satiated cod showed a diminished appetite and the reduced food intake may be one of the reasons for the failure to maintain a high glycogen density in the liver. Another possible explanation is that satiation leads to less efficient absorption of carbohydrates from the diet.

The present study was begun with the hope of establishing suitable criteria for a normal cod in terms of hematocrit, plasma *p*H, plasma proteins and glucose levels. In mammals, these parameters have a narrow range of variation at homeostasis and any large deviation may be readily related to an abnormal physiological condition. On the other hand, the blood properties of a marine teleost differ in many aspects from those of terrestrial, and even freshwater, vertebrates owing to the peculiarities inherent in adapting to a hypertonic environment. The small amount of water lost by excretion will have to be replaced by extraction from the environment. This is accomplished in the marine teleost by oral ingestion of sea water, followed by isotonic absorption through the intestines and excretion of salts by an obscure mechanism in the gill area (Brown and Stein, 1960). In an advanced state of starvation in the cod, reduced levels of plasma protein and glucose render the blood more hypotonic. Osmoregulation, however, does not seem to proceed as expected, simply by lowering extrarenal salt excretion and thereby even saving some osmotic work. The cod showed definite evidence of dehydration and consequent decrease in total blood volume. Thus, there was an apparent increase in hematocrit in depletion II, despite considerable loss in body weight during fasting. Plasma protein and glucose also reached apparently higher levels in some fish. While the mean value for protein was not appreciably altered by starvation, glucose showed a decrease in the average value in depletion I and II. These results reflect differences in the rates of synthesis, turnover, and utilization of erythrocytes, protein, and glucose. The use of these parameters

TABLE IV. Typical record of one male and one female cod during feeding, fasting, and liver regeneration.

Physiological condition	Body wt (*kg*)	Hematocrit (%)	Plasma *p*H	Plasma protein (% w/v)	Plasma glucose (*mg/100 ml*)	Liver wt (*g*)	Liver wt (*% body wt*)	Liver glycogen (*mg/100 g wet wt*)
Male								
Feeding	1.33	–	–	5.23	122			
Satiated	–	28.8	7.24	5.05	95			
Depletion I	1.16	31.8	7.49	5.10	70			
Depletion II	1.10	34.5	7.50	7.04	92			
Regeneration	1.50	32.5	7.28	9.10	129	54.1	3.60	1632
Female								
Feeding	1.19	–	–	6.33	122			
Satiated	–	25.0	7.24	6.38	129			
Depletion I	0.96	21.3	7.49	3.75	93			
Depletion II	0.93	26.8	7.56	7.39	67			
Regeneration	1.25	27.0	7.16	8.75	127	54.6	4.37	960

as indices for the evaluation of the nutritional status of cod presents difficulties on account of the apparent reversal of the trend with prolonged starvation, and the normally wide range of distribution of these factors.

The steady increase in alkalinity of blood plasma with the length of fasting period may be due to extensive deamination of protein amino acids for gluconeogenesis. Plasma *p*H rose from 7.24 to 7.54 during 5 weeks of fasting, but rapidly returned to normal when feeding was resumed. There was no noticeable change in the free polysaccharide content of plasma during fasting, feeding, and liver regeneration despite the changes in liver glycogen density. This supports the belief that liver is the site of degradation of carbohydrates in the cod, despite its limited ability to maintain a glycogen reserve. It is not known whether the failure to store glycogen is the result of a genic alteration during the process of evolution of the species, or simply an adaptative change caused by the available diet (high fat content in this case) and may be reversed by a high carbohydrate diet.

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Use of Overhead Cover by Rainbow Trout Exposed to a Series of Light Intensities[1]

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ABSTRACT

Fingerling and yearling trout (*Salmo gairdneri* Richardson) responded differently when exposed to a series of artificial daylight illuminations. Fingerling trout showed no apparent response to the overhead cover, being randomly distributed at all light intensities. Yearling trout, except in total darkness, exhibit a positive response to the overhead cover, but their pattern of behaviour differed with the light intensity and the positioning of the cover.

INTRODUCTION

Light is of great importance in the lives of fishes. Not only do the intensities and wavelengths perceived by their eyes vary according to species, but the nature of the reaction may exert a strong influence on the physiology and behaviour of the fish (Hoar et al., 1957; Hoar, 1958; Ivlev, 1960; Nikolsky, 1963).

This paper reports on the use of overhead shade by fingerling and yearling rainbow trout, *Salmo gairdneri* Richardson, and the general behaviour of these fish when exposed to a series of intensities of artificial daylight.

MATERIALS AND METHODS

The series of experiments was conducted in the underground fisheries laboratory of the University of Guelph where an accurate control of the environment was possible.

The basic apparatus consisted of an overhead light source and a round, white-bottomed plastic tank measuring 1.83 m in diameter. The water level was maintained at a depth of 15 cm and held at 11–12 C by cooling coils located beneath the plastic bottom of the tank. The water in the tank was replaced after each experiment. Illumination was provided by a battery of G.E. incandescent daylight bulbs at a series of test intensities of total darkness, 10^{-3} ft-c, 1.0 ft-c, and 10×2 ft-c measured by a photoelectric cell aimed vertically upwards while 10 cm below the surface of the water. The source of illumination was hidden from the fish by means of an opal glass and white cloth screen which allowed only overhead diffused light to reach the surface of the tank.

Fingerling and yearling rainbow trout used in the study were reared from eggs of the spring-spawning Winthrop strain donated by the United States

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Fish and Wildlife Service from the National Fish Hatchery at Manchester, Iowa. The eggs were hatched in a Heat Incubator in darkness and reared under a continuous illumination of 5.0 ft-c provided by overhead F40-WW Sylvania fluorescent lights. By this procedure all fish used in the study were similarly acclimated to light, taking into account that neither the retinal epithelial pigment nor the rods and cones possess a diurnal rhythm in their position under conditions of continuous light (Ali et al., 1961).

Overhead shade or cover was provided in each of the two following ways: (i) by covering a quadrant of the tank with the apex of the pie-shaped cover towards the center of the tank, and (ii) by covering a quadrant of the tank with the apex of the cover towards the periphery of the tank. The cover was floated in the surface of the water in both cases.

In each experiment observations were made on the movement and distribution of the fish for a 120-min period after placing them in the tank by means of (i) 35-mm photographs, using an electronic flash at all test light intensities and taken at 10-min intervals during the first 60 min and at 15-min intervals during the second 60-min period, (ii) 16-mm motion pictures at the higher light intensities, and (iii) visual observation under conditions other than total darkness. Each test run was made with 20 fingerling or yearling trout taken randomly from a stock of several hundred fish.

Data taken from the photographic records for each experiment were analyzed by the Chi-square method and corrected by combining the probabilities from significance tests (Bartlett, 1964). The use of electronic flash under conditions of total darkness and low light intensity caused, at most, a momentary response by the fish which was considered to be insignificant. At the intermediate and high light intensity there was no detectable reaction to the electronic flash.

RESULTS

Quadrant Cover with Apex Towards Center of Tank

Data for the distribution of fingerling and yearling trout exposed to a series of artificial daylight intensities are given in Table I.

IN TOTAL DARKNESS

An analysis of the distribution data showed both the fingerling and yearling trout to be randomly distributed over the bottom of the tank with no reference to the overhead cover when held in total darkness.

An inspection of the physical attitude of the fish of both ages revealed that they were typically in a resting position, lying more or less inactive near the bottom of the tank (Fig. 1).

AT A LOW OVERHEAD LIGHT INTENSITY

An analysis of the time-lapse photographs taken at the low light intensity of 10^{-3} ft-c showed the fingerling trout to be randomly distributed while swimming continuously about the tank. However, the yearling were found to show a significant preference for the covered quadrant.

TABLE I. A summary of observations on the distribution of fingerling and yearling trout on the open area of the quadrant-covered tank during a 120-min period of different light intensities. Corresponding χ^2 and P values were for differences between open and covered areas.

	Light intensity (*ft-c*)															
	0				10^{-3}				1.0				2×10			
	Fingerling		Yearling		Fingerling		Yearling		Fingerling		Yearling		Fingerling		Yearling	
Observation numbers	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1	17	15	13	15	16	13	0	0	17	13	0	0	13	9	1	0
2	16	16	14	10	16	12	0	0	12	11	0	0	12	12	1	0
3	19	18	12	11	18	13	0	0	9	12	0	0	14	10	3	0
4	17	18	11	16	13	7	0	0	16	11	0	0	12	11	3	0
5	17	15	12	15	13	15	0	0	10	12	0	0	17	15	0	0
6	15	17	15	10	15	13	0	0	13	11	0	0	15	16	0	0
7	17	17	15	8	8	14	0	0	10	15	3	0	10	13	0	2
8	18	18	12	13	12	17	0	1	12	9	0	0	10	14	1	1
9	16	16	13	16	12	16	0	0	11	13	1	0	12	12	2	1
10	15	18	14	16	13	17	0	1	14	17	0	0	10	13	0	0
χ^2	3.93		4.75		2.98		27.63		6.44		27.63		6.44		27.63	
P	>0.40		>0.30		>0.60		<0.001*		>0.20		<0.001*		>0.20		<0.001*	

*P-values marked with an asterisk were significant at 0.05 level.

On being placed in the tank, the fingerlings swam actively without specific direction and showed no recognition of the overhead cover. The yearlings, by contrast, swam less actively, and within an average time of approximately 3 min showed a strong preference for the covered part of the tank. For the remainder of the 120-min study period, the yearlings typically stayed beneath the cover, a fish occasionally moving out for a few seconds, then quickly returning beneath the cover.

AT AN INTERMEDIATE LIGHT INTENSITY

At the intermediate test light intensity of 1.0 ft-c, the fingerling trout were randomly distributed in the tank over the 120-min study period. The yearling trout, however, showed an immediate significant preference for the covered area, moving beneath the cover within a minute after being placed in the tank. After approximately 20 min some fish would appear along the inner border of the cover, and frequently one or two fish would swim into the open area (Fig. 1). Gradually, the number of fish moving between the covered and open areas of the tank increased although the preference for the covered area continued to be significant.

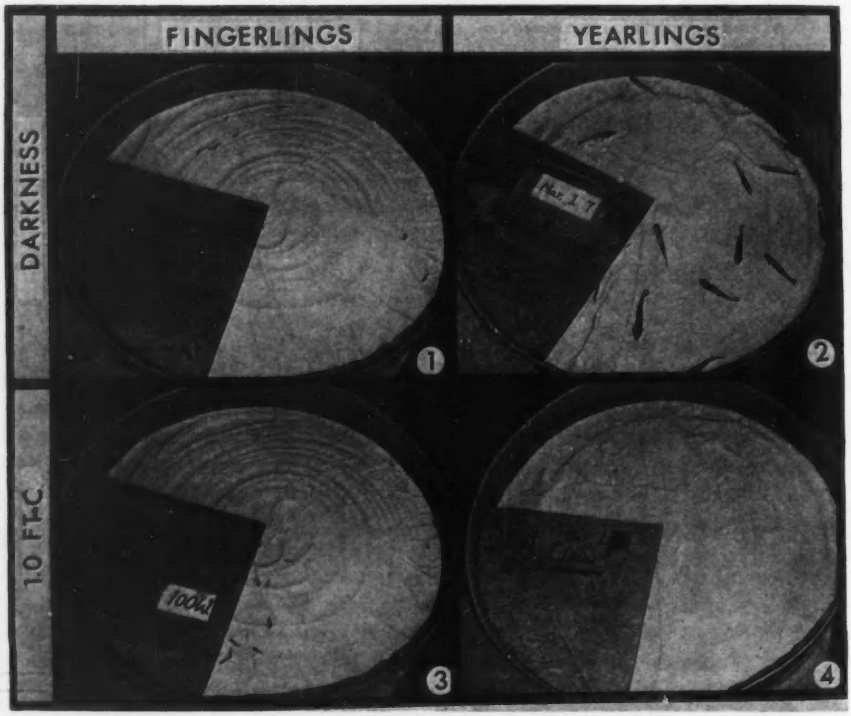

FIG. 1. The distribution of fingerling and yearling trout held in total darkness and at an intermediate illumination of 1.0 ft-c with apex of overhead cover directed to center of tank.

AT A HIGH LIGHT INTENSITY

The fingerling trout held at 2 × 10 ft-c showed no preference for either the open or covered areas of the tank, moving in and out of the covered area as they swam actively about the tank. The fingerlings exhibited a noticeable preference for the edge of the tank.

The yearling trout showed an immediate and positive response to the covered area when placed in the tank. After a few minutes, fish occasionally moved out into the open but stayed there only momentarily, the preference of the yearlings for the covered area being highly significant. At this high light intensity the yearling trout moved with obvious activity.

Quadrant Cover with Apex Towards Edge of Tank

Data for the distribution of fingerling and yearling trout exposed to a series of artificial light intensities are given in Table II.

IN TOTAL DARKNESS

Under the condition of total darkness, both the fingerling and yearling trout were randomly distributed over the bottom of the tank at all times without reference to the overhead cover. A study of the attitudes of the fish in the photographs (Fig. 2) suggested that both fingerlings and yearlings were generally inactive, resting quietly near the bottom.

AT LOW OVERHEAD LIGHT INTENSITY

On being placed in the tank at an overhead light intensity of 10^{-3} ft-c, the fingerling trout began to swim actively. Within a few seconds, without specific direction or apparent reference to the overhead cover, the distribution of the fingerlings was random during the 120-min study period.

The yearling trout, on introduction into the tank, swam slowly under the covered area within 1–3 min. For the duration of the study period, the yearlings stayed typically under the cover. There was frequent movement of yearlings from under the cover although those fish venturing forth, seldom more than one or two fish at a time, returned to cover after a few seconds. The electronic flash was observed to accelerate the movement of the yearling fish towards cover but had no effect on the fingerlings.

TABLE II. A summary of observations on the distribution of fingerling and yearling trout on the open area of the reversed quadrant-covered tank during a 120-min period of different light intensities. Corresponding χ^2 and P values were for differences between open and covered areas.

	Light intensity (*ft-c*)															
	0				10^{-3}				1.0				2×10			
	Fingerling		Yearling		Fingerling		Yearling		Fingerling		Yearling		Fingerling		Yearling	
Observation numbers	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1	16	14	16	15	13	13	2	1	18	15	1	0	10	15	0	0
2	15	10	17	14	11	15	0	1	16	13	1	2	14	18	2	0
3	17	12	17	17	12	15	1	1	17	17	1	12	14	14	11	2
4	16	13	13	17	15	15	2	1	15	16	3	10	10	17	18	9
5	15	15	14	15	17	17	1	1	10	17	4	8	18	16	11	5
6	13	18	15	15	18	12	1	1	18	18	11	10	13	16	19	12
7	17	17	14	17	15	18	4	11	16	14	3	14	11	17	14	9
8	13	17	13	13	16	16	7	9	17	15	2	17	16	16	18	11
9	14	15	12	12	14	17	11	5	16	14	3	18	16	14	19	11
10	12	14	11	15	16	14	8	10	11	14	2	17	16	12	20	4
χ^2	0.54		0.66		0.54		27.63		0.66		15.65		1.35		16.22	
P	>0.95		>0.95		>0.95		<0.001*		>0.95		<0.005*		>0.80		>0.001*	

*P-values marked with an asterisk were significant at 0.05 level.

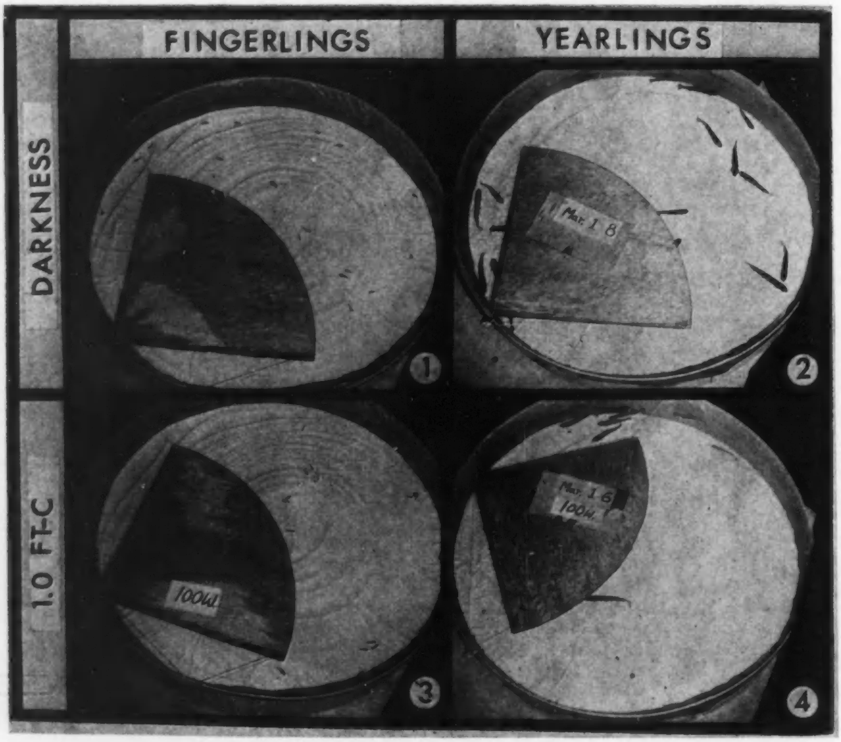

FIG. 2. The distribution of fingerling and yearling trout held in total darkness and at an intermediate illumination of 1.0 ft-c with apex of overhead cover directed toward periphery of tank.

AT INTERMEDIATE OVERHEAD LIGHT INTENSITY

At the intermediate test intensity of 1.0 ft-c the fingerling trout were randomly distributed in the tank. Their movement about the tank was more-or-less continuous and appeared to be nondirectional.

The yearling trout, by contrast, selected the covered area of the tank and remained there for approximately 10 min. After this time, there was a tendency for the fish to venture into the open area of the tank (Fig. 2).

AT HIGH LIGHT INTENSITY

The fingerling trout were randomly distributed in the tank at the high test intensity of 2 × 10 ft-c. The fish swam actively between the open and covered areas with no apparent recognition of the overhead cover.

All yearling trout, on being placed in the tank, moved directly to beneath the covered area and remained there for 10–20 min before they appeared again in the open area of the tank. After approximately 40 min, movement between the covered and open areas was a common occurrence.

DISCUSSION

Observations made on fingerlings and yearling trout exposed to the series of artificial daylight illuminations showed differences in the response of each to the overhead cover at all of the test light intensities, except that of total darkness. It was found that the fingerling trout showed no preference for either the covered or open areas of the tank, being randomly distributed at all of the test light intensities. The yearling trout, however, demonstrated a significant preference for the covered portion of the tank under all test light intensities except when randomly distributed in total darkness. The reaction of the yearling trout varied with the light intensity when first put in the tank, the movement to beneath the cover being more immediate at the higher rather than the lower light intensity. Also, their subsequent movement between the covered and open areas of the tank was more pronounced at the higher light intensities.

These observed differences in response to light with age are in agreement with the results of several workers concerned with other aspects of fish behaviour and physiology. Nikolsky (1963) stated that the reactions of fish to light changed in ontogeny. From histophysiological studies, Ali (1959) reported that the retinomotor responses of the Pacific salmons (*Oncorhynchus*) became more and more marked with age. Hoar (1958) stated that *Oncorhynchus* spp. are at first less photonegative but, as they grow older, the photonegative response becomes more dominant. Although the present experiment measured a different response in the rainbow trout, there seems to be uniformity in the thesis that visual acumen differs with age.

The selection of overhead cover by the yearling trout, as recorded under the laboratory conditions outlined in this paper, conforms generally to observations made on the ecological relationship of fish with cover under natural environmental conditions as reported by several workers (Hoar, 1958; Hartman, 1963). The tendency of the fingerling trout to prefer the edge of the tank at the higher light intensities in the present study would seem to be explained as a form of cover selection.

The more significant findings of the study are summarized graphically in Fig. 3. Apparent is the absence of a relationship between the distribution of fingerling trout within the tank and light intensity without respect to the position of overhead cover. Yearling trout, however, not only showed a positive response to the cover, but responded in a different manner depending on whether the apex of the cover was directed towards the center or the edge of the tank. The percentage of yearling trout staying under the cover during the 120-min study period (except in total darkness) was lower when the apex of the cover was directed towards the edge than when it was directed towards the center of the tank. This would suggest that the presence of a length of the side wall of the tank under the cover made the covered area more attractive to the yearling trout.

Harden Jones (1956) found that among minnow species which he studied, there was a tendency to avoid bright light, particularly if the light–dark

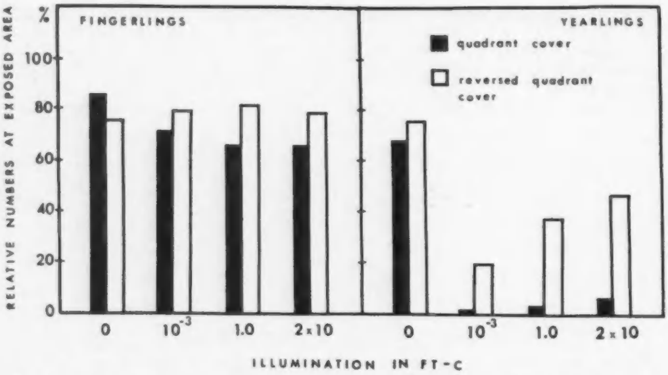

FIG. 3. A comparison of the response to overhead cover by fingerling and yearling trout under different intensities of artificial daylight.

boundary was sharp. The photometric measurement of light intensities in the covered and open areas of the tank in the present experiment (Fig. 3) revealed a less pronounced demarkation between the dark and light zones at the higher light intensities which may account for a greater tendency of the yearling trout to roam from beneath the cover with increasing light intensity. However, it does not explain the observed behaviour of the yearlings, when first placed in the tank, to dash more quickly under the cover at the higher light intensities than at the low intensity of 10^{-3} ft-c. From the fact that the yearling trout were randomly distributed in total darkness, it would seem that the observed preference of these fish for the covered area of the tank under the various levels of illumination was a phototaxic rather than a tactual response.

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Some Commercial Atlantic Herring Oils; Fatty Acid Composition[1]

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ABSTRACT

Twelve commercial samples of oil from Atlantic herring (*Clupea harengus* L.) were examined for fatty acid composition by gas–liquid chromatography. Individual fatty acid components varied widely between these oils, but an assessment of the respective totals of saturated, monounsaturated, and polyunsaturated acids from these oils and from oils of other species has led to an empirical formula giving the total per cent polyunsaturated fatty acids in marine oils as:
total polyunsaturated fatty acids = 10.7 + 0.337 (iodine value oil — 100)

In the Atlantic herring oils studied the average proportions of the saturated, monounsaturated, and polyunsaturated fatty acids were 20, 60, and 20%, respectively. In comparison with herring oil analyses reported from other areas the total saturated acids in the Atlantic herring oils are slightly lower, but there is general agreement that hexadecanoic acid averages about 60% of the total saturated fatty acids. Relationships which are fairly consistent among other acids and apparently not related to iodine value include the ratio of hexadecanoic acid to hexadecenoic acid plus octadecenoic acid, the ratio of C_{20} and C_{22} successor acids to C_{18} linoleic-type precursor acids, and the ratio of linolenic-type acids compared to immediate precursors of the same chain length. As iodine value increases the saturated acids increase slightly, and in the linolenic type acids the ratio of C_{20} to C_{18} fatty acids increases more noticeably than the ratio of C_{22} to C_{18}. The ratio of C_{22} to C_{20} linolenic-type acids accordingly decreases with increasing iodine value. The ratio of total linolenic-type acids to total linoleic-type acids apparently increases with increasing iodine value. Comparisons are made with oil analyses of clupeids taken in other oceanic areas.

INTRODUCTION

The importance of the herring (*Clupea harengus L.*) in the Northwest Atlantic fisheries has never reached that of the related species (*Clupea pallasii* V.) on the Pacific coast of Canada. In 1961, for example, production of herring oil on the Atlantic coast was only 1.1 million lb as against 41.7 million lb for the Pacific coast. A considerable expansion of the herring fishery around the Maritime Provinces and in Newfoundland is, however, currently being undertaken. Since there has been no recent examination of the properties of commercial Atlantic herring oils the present study was undertaken with some available samples to obtain a general picture of the range of fatty acid compositions likely to be encountered in current and future production.

An economic survey of the production of herring oil in the Atlantic provinces has recently been carried out (Davis, MS, 1965) which includes a review of current production methods and yields of meal and oil, with a survey of existing plants and of projected plants. Standard texts (Bailey, 1952; Tressler and

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Lemon, 1951) and a detailed report (Jul and Kondrup, 1953) describe herring utilization and the technology of herring meal and oil production. The publications of the Fisheries Research Board of Canada in recent years on fisheries by-products have been reviewed (Ackman and Jangaard, 1964).

Herring are subject to large fluctuations in fat content (approximate range 4–20%) which has locally been the subject of detailed studies (Leim et al., 1957; Leim, 1958), and was also studied by Lovern (1938, 1942) from the point of view of fatty acid composition of the oil from North Sea herring. Iles and Wood (1965) have discussed the fat–water relationship in North Sea herring, and Linko (1964) has compared the neutral fat and phospholipid fatty acids of Baltic herring. The fishery, migration, biology, and reproduction of herring in this area are the subject of recent detailed reports (Leim et al., 1957; Leim, 1958; Scattergood and Tibbo, 1959; McKenzie and Tibbo, 1961; Olsen, 1961) and similar data on Pacific herring have recently appeared (Taylor, 1964). Lambertson and Braekkan (1965) have recently surveyed the fatty acid composition of a number of clupeids from different oceans. The samples for the present study are not in general correlatable with the chief factors responsible for variations in fat and fatty acid composition, namely, the size and sexual maturity of the herring from which they were processed, but are sufficient in number and variation in composition to permit some interesting general conclusions to be drawn. Included in some comparisons are recent analyses of Pacific herring oils, of European oils, and of a high iodine value oil from the Pacific pilchard (*Sardinops sagax* (Jenyns) or *Sardinops caerulea* (Girard)).

EXPERIMENTAL

SAMPLES

Numbers 1, 5, 6, and 12 were personally collected from production lines in 1964 by W. R. Davis, who also donated samples 2, 8, and 11. Other samples were purchased from or donated by various firms. The British Columbia herring oil was purchased in 1960 for use in studies on segregation of marine oil methyl esters (Jangaard, 1965). Details of the origin of the samples are as follows:

No. 1. Caraquet, N.B. Produced in August 1964. Pure herring oil from whole fish.

No. 2. "Summerside, P.E.I.'" No other data.

No. 3. Black's Harbour, N.B. Produced in November 1964. Sold as "Good" oil (low free fatty acid). Produced from herring 5–8 inches long, but sample largely from scrap and waste from sardine processing.

No. 4. Chloroform extract of body only. Mature Nova Scotia herring, origin unknown.

No. 5. Short Beach, Yarmouth Co., N.S. Produced in August 1964. In addition to whole herring some oil from groundfish offal may have been included.

No. 6. Shippegan, N.B. Produced in August 1964. Pure herring oil from whole fish.

No. 7. As in No. 3. Sold as "Industrial" oil (high free fatty acid).

No. 8. Sample from Black's Harbour, N.B. No other data.

No. 9. Commercial herring oil. Stated to be from Shippegan, N.B., produced in late 1964.

No. 10. As in No. 9, different drum.

No. 11. Sample marked "St. Andrews." No other data.

No. 12. Black's Harbour, N.B. Produced in July 1964. Stated to be pure herring oil from whole fish somewhat above sardine size.

ANALYSES

The samples had, in some cases, already been alkali refined. The free fatty acid contents have therefore not been determined. Iodine values were determined with Wijs reagent. The nonsaponifiable material was determined by the Official Method of the American Oil Chemists' Society. The fatty acids recovered from this procedure were esterified with boron trifluoride–methanol (Metcalfe and Schmitz, 1961). Gas–liquid chromatographic analyses were carried out primarily on a Barber-Colman Model 10 apparatus, with two columns (glass) 6 ft long and 3 mm I.D., packed with 15% EGSS-Y and 12% EGSS-X organosilicone polyesters. Identifications of minor components are provisional (Ackman and Burgher, 1963, 1964). A Model 5121 flame ionization detector was employed with mechanically integrated recorder areas corrected to give weight per cent composition (Ackman and Sipos, 1964a). The data in Table I are given to two places of decimals solely to show the relatively small proportion of some components. The probable errors are estimated as <5% for major components, <10% for low-to-medium range components, but up to 50% for very minor components. In some cases of unusual composition auxiliary studies were carried out to confirm the identity or purity of certain components. Branched chain fatty acids have been included in the corresponding major components. Sample No. 4 was studied in detail for these acids (Ackman and Sipos, 1965) and it was found that an unknown material, tentatively identified as phytanic (3,7,11,15-tetramethylhexadecanoic) acid methyl ester, could lead to up to 100% overestimation of 17:0.[2] The values given in Tables I and II are, however, uncorrected.

RESULTS AND DISCUSSION

GENERAL COMPOSITION

It will be noted that the iodine values calculated from the gas–liquid chromatographic analyses (Table I) are 5–10 units lower than the Wijs iodine values determined on the oils. The probable reason for this is the presence of polymers formed through oxidation of the unsaturated fatty acids during the commercial processing of the herring. These polymers (Fukuzumi and Maruyama, 1965) would be nonvolatile and not appear in the gas-liquid chromatograms. Since such polymers most probably involve the polyunsaturated fatty acids

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[2] Shorthand notation for chain length:number of double bonds. With addition of ωx (e.g. 18:3ω3) the number of carbon atoms from the center of the ultimate double bond to the terminal methyl group defines a definite unsaturated acid of the normal methylene-interrupted type.

TABLE I. Properties of some Atlantic herring and two other oils with weight per cent compositions of the fatty acid methyl esters derived from the oils.

		Atlantic herring oils												Pacific herring oil (This study)	Pacific pilchard oil (Ackman and Sipos, 1964b)
Sample No.:		1	2	3	4	5	6	7	8	9	10	11	12		
Oil iodine value (Wijs):		111.6	121.4	124.8	124.6	128.0	123.6	129.8	131.6	129.8	130.8	138.3	139.2	129.8	192
Per cent nonsaponifiable:		0.85	0.74	1.17	0.74	0.80	1.74	1.74	0.91	1.35	1.09	1.19	0.78	0.60	0.69
Refractive index ($n^D 25$):		1.47107	1.47211	1.47225	1.47307	1.47273	1.47220	1.47314	1.47284	1.47303	1.47275	1.47425	1.47389	1.47282	–
Fatty acid[a]	Double bond positions[b]	Weight per cent fatty acid methyl ester compositions[e,f]													
12:0	–	0.07	0.09	0.13	0.06	0.10	0.10	0.11	0.05	0.11	0.13	0.09	0.11	0.08	–[d]
14:0	–	5.11	6.01	8.36	4.60	6.90	5.90	7.91	6.42	5.07	4.87	7.58	8.15	5.66	7.6
14:1	9[c]	0.34	0.27	0.30	0.24	0.45	0.44	0.28	0.23	0.39	0.46	0.20	0.33	0.20	TRA
15:0	–	0.32	0.39	0.40	0.28	0.28	0.52	0.39	0.30	0.36	0.42	0.23	0.39	0.25	0.6
15:1	9[c]	0.04	0.09	0.05	0.05	0.04	0.10	0.05	0.07	0.15	0.14	0.06	0.09	0.08	TRA
16:0	–	10.10	11.84	11.88	10.55	11.59	12.79	12.22	12.72	10.89	10.26	15.00	12.50	16.64	16.2
16:1	9[c]	10.19	9.32	6.30	11.56	7.87	7.00	6.30	8.80	12.01	11.63	8.94	8.50	7.63	9.2
16:2	9,12	0.68	0.48	1.02	0.78	0.93	0.41	0.80	0.68	0.70	0.85	0.73	0.72	0.79	1.3
16:3	6,9,12	0.27	0.27	0.59	0.47	0.44	0.20	0.53	0.48	0.58	0.52	0.62	0.67	0.39	1.0
16:4	6,9,12,15	0.62	0.89	0.29	0.89	0.78	0.21	0.37	0.50	1.15	1.12	0.59	0.81	0.54	1.2
17:0	–	0.11	0.22	0.20	0.21	0.13	0.22	0.29	0.19	0.21	0.29	0.20	0.18	0.27	0.7
17:1	9[c]	0.24	0.13	0.18	0.12	0.14	0.30	0.28	0.12	0.12	0.30	0.14	0.15	0.29	0.2
18:0	–	0.68	1.00	0.73	0.82	1.07	2.13	1.20	0.94	1.16	1.12	1.30	0.77	1.79	3.5
18:1	9[c]	9.31	13.01	11.09	10.18	12.07	21.36	11.86	12.74	12.63	12.53	13.60	9.86	22.69	11.4
18:2	9,12	0.59	0.98	1.35	0.58	1.32	2.88	1.47	1.14	0.74	0.58	1.15	1.08	0.55	1.3
18:3	6,9,12	0.05	0.06	0.04	0.14	0.04	0.09	0.04	0.07	0.09	0.10	0.15	0.11	0.08	TRA
18:3	9,12,15	0.37	0.60	0.76	0.16	0.79	0.80	1.08	0.55	0.28	0.36	0.67	0.58	0.43	0.9
18:4	6,9,12,15	1.24	1.71	2.38	1.14	2.19	1.10	2.54	1.69	1.53	1.47	2.30	2.50	1.63	2.0

19:0	–	NSA	NSA	NSA	NSA	NSA	TRA	NSA	NSA	NSA	NSA	NSA	NSA	TRA	0.02
19:1	9[c]	0.05	0.09	0.11	0.10	0.06	0.05	0.08	0.05	0.06	0.12	0.07	0.04	0.10	TRA
19:4	?	TRA	TRA	TRA	TRA	TRA	TRA	TRA	NSA	NSA	NSA	TRA	TRA	NSA	–[d]
20:0	–	NSA	NSA	NSA	NSA	NSA	TRA	NSA	NSA	NSA	TRA	NSA	NSA	TRA	TRA
20:1	11[c]	19.94	17.08	13.49	16.78	15.16	13.00	15.07	14.10	16.12	15.91	10.99	14.93	10.68	3.2
20:2	11,14	0.18	0.20	0.25	0.13	0.17	0.31	0.25	0.18	0.14	0.16	0.16	0.14	0.16	TRA
20:3	5,8,11	NSA	NSA	NSA	NSA	NSA	NSA	NSA	NSA	NSA	NSA	NSA	NSA	NSA	TRA
20:3	8,11,14	0.21	0.17	0.14	0.09	0.14	0.26	0.18	0.16	0.08	0.12	0.11	0.11	0.12	0.1
20:4	5,8,11,14	0.18	0.35	0.23	0.15	0.32	0.53	0.42	0.33	0.40	0.46	0.34	0.20	0.38	1.1
20:4	8,11,14,17	0.26	0.33	0.52	0.39	0.47	0.70	0.65	0.53	0.42	0.36	0.72	0.76	0.60	0.5
20:5	5,8,11,14,17	3.89	5.70	5.56	8.19	5.86	5.13	5.22	8.42	7.39	8.34	8.83	8.53	8.14	16.9
21:5	?	0.26	0.25	0.46	0.28	0.50	0.32	0.43	0.54	0.33	0.38	0.55	0.59	0.61	0.6
22:1	11[c]	30.57	22.18	25.95	25.62	23.41	14.75	21.61	20.84	19.82	20.58	16.93	19.29	11.96	3.8
22:4	7,10,13,16	NSA	NSA	TRA	NSA	TRA	NSA	TRA	TRA	NSA	NSA	TRA	TRA	TRA	0.1
22:5	4,7,10,13,16	0.12	0.14	0.19	0.16	0.30	0.28	0.23	0.18	0.41	0.36	0.22	0.23	0.17	0.2
22:5	7,10,13,16,19	0.60	0.47	0.60	0.49	0.72	1.30	0.56	0.77	1.13	1.21	0.76	0.77	0.75	2.3
22:6	4,7,10,13,16,19	2.02	4.81	5.15	3.58	4.50	5.27	6.20	4.86	3.87	4.55	4.95	5.32	4.83	12.9
24:1	?	0.83	0.86	0.75	0.72	0.80	0.91	0.79	0.76	0.36	0.24	1.21	0.88	0.94	0.5
24:5	?	0.42	0.24	0.43	0.35	0.52	0.41	0.48	0.40	0.47	–[d]	0.37	0.54	0.34	0.2
24:6	?	0.05	0.09	0.09	0.11	0.21	0.16	0.12	0.13	0.29	–[d]	0.14	0.13	0.14	0.1
Iodine value calc from composition:		99.2	116.1	117.4	119.7	119.9	120.7	122.5	126.1	126.5	127.9	128.3	131.0	121.9	190

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[a] Shorthand notation for chain length: number of double bonds.
[b] From carboxyl group.
[c] Other isomers may be present.
[d] Analysis for these materials not carried out.
[e] NSA = no significant amount present; TRA = trace.
[f] Branched chain fatty acids included in major components.

TABLE II. Chain length compositions of some Atlantic herring oils, of some other herring oils, and of pilchard oil.

		Atlantic herring oils												Other herring oils			Pacific Pilchard Oil
														Pacific		European	
Sample No.:		1	2	3	4	5	6	7	8	9	10	11	12	(This study)	(Gruger et al., 1964)	(Klenk and Eberhagen, 1962)	(Ackman and Sipos, 1964b)
Fatty acid chain length	Averages No. 1-12 only	Weight per cent different chain lengths[c]															
C_{12}	0.10	0.07	0.09	0.13	0.06	0.10	0.10	0.11	0.05	0.11	0.13	0.09	0.11	0.08	-	-	-
C_{14}	6.73	5.45	6.28	8.66	4.48	7.35	6.34	8.19	6.65	5.46	5.33	7.78	8.48	5.86	7.6	6	7.6
C_{15}	0.43	0.36	0.48	0.45	0.33	0.32	0.62	0.44	0.37	0.56	0.51	0.29	0.48	0.33	0.4	+	0.6
C_{16}	22.78	21.86	22.80	20.08	24.25	21.61	20.62	20.22	23.18	25.33	24.38	25.88	23.20	25.99	27.6	19	28.9
C_{17}	0.39	0.35	0.35	0.38	0.33	0.27	0.52	0.57	0.31	0.33	0.59	0.34	0.33	0.56	0.5	+	0.9
C_{18}	17.23	12.24	17.36	16.35	13.02	17.48	28.36	18.19	17.13	16.43	16.16	19.17	14.90	27.17	24.1	26	19.1
C_{19}	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C_{20}	23.19	24.66	23.83	20.19	25.73	22.12	19.93	21.79	23.72	25.09	25.35	21.15	24.67	20.08	18.4	21	21.8
C_{21}	0.41	0.26	0.25	0.46	0.28	0.50	0.32	0.43	0.54	0.33	0.38	0.55	0.59	0.61	-	+	+
C_{22}	27.40	33.31	27.60	31.89	29.85	28.93	21.60	28.60	26.65	25.23	26.70	22.86	25.61	17.71	20.5	24	19.3
C_{24}	1.36[a]	1.30	1.19	1.27	1.18	1.53	1.48	1.39	1.29	1.12	0.24[b]	1.72	1.55	1.42	0.9	1	0.8

[a] For eleven samples.

[b] Polyunsaturated omitted.

[c] + signifies present in small amounts.

of high iodine value even a very small amount of polymer will result in a significant decrease in iodine value calculated from GLC data in comparison with the Wijs value for the oil (or esters) where the unreacted double bonds in the polymers will still be determined. In addition, oxidation or other losses may occur during preparation of the esters (Ackman and Burgher, 1964). The effects of oxidation on refractive index have been noted (Bailey, 1952). In essence then, the gas–liquid chromatographic data should be regarded as the analysis of an oil of slightly different composition from the original sample. The samples have therefore been numbered and arranged in the tables in order of the iodine values calculated from the ester analyses rather than in the order of original iodine values.

The detailed analyses of Atlantic herring oils in Table I are perhaps remarkable for illustrating that with one species from one area any one component fatty acid can sometimes show drastic variations in spite of general trends in relation to iodine value. The same observation may be made with the data of DeWitt (1963) for cod liver oils. Considering the saturated acids of the herring oils as iodine value increases, there is little evidence for systematic change in proportions of 14:0, 16:0, or 18:0. This is also probable with the monounsaturated acids for 16:1 and 18:1, but with 20:1 there is some general tendency for the proportion to drop as iodine value increases, and this is even more marked in the 22:1. With the major polyunsaturated acids 20:5ω3 shows general evidence for an increase with increasing iodine value, and this may also be true of the 22:6ω3. However, the individual variations are very marked.

In considering these relationships the notable difference in Sample No. 1 is clear. The origin of this sample as pure herring oil is definite and although the oil iodine value of 111.6 is very low it is reported that oil from the same plant produced in 1965 has iodine values as low as 95 (Lawson, personal communication). The significance to be attached to the single analysis of British Columbia herring oil, as shown by the variations in the local samples, is not very great. However, the proportions of 16:0 and 18:1 are definitely higher than that of the local samples, while 20:1 and 22:1 are lower, although it should be noted that the total monounsaturates are in the same range (see below). In the case of the high iodine value pilchard oil, as compared to the Atlantic herring oils, 14:0 does not differ significantly, 16:0 and 16:1 are somewhat higher, and 18:1 is about the same, with the high iodine value originating largely in the replacement of 20:1 and 22:1 by 20:5ω3, 22:5ω3, and 22:6ω3.

The chain length data for the local Atlantic herring oils (Table II) also show such wide individual variations that only generalizations are possible. It appears that the C_{16} acids increase slightly with increasing iodine value, the C_{18} and C_{20} chain lengths are more or less constant, and the C_{22} chain length decreases somewhat. Two analyses of Pacific herring oils are included for comparison, one being from the present study. Comparison with the average values for the local Atlantic herring oils suggests that in the Pacific herring the C_{14} chain length is about the same, the C_{16} and C_{18} chain lengths are higher, especially the latter, and the C_{20} and C_{22} chain lengths lower, more especially the C_{22}. A herring oil from European waters (iodine value for methyl esters 119) is smaller in the C_{14} chain length, lower in the C_{16}, much higher in the C_{18}, and somewhat lower in the C_{20} and C_{22} chain lengths. However, undue emphasis cannot be placed on these analyses. The high iodine value pilchard oil shows a progressive shift in chain lengths in comparison with the average for the local Atlantic herring oils, from higher to lower proportions as the chain length increases.

To overcome the problem of interpreting the detailed GLC analyses or the chain length analyses where erratic values for single components cause difficulties the data from Table I and some literature data have been broken down into totals for different types of acids irrespective of chain length, and of ratios of various types of structurally related acids (Table III). Some results similar to those obtained by DeWitt (1963) with cod liver oils are obtained with the local Atlantic herring oils for the three main classes of acids, the saturated, monounsaturated, and polyunsaturated. The total saturated acid levels in these samples vary more or less randomly, although there is probably some slight increase with iodine value. The monoethylenic and polyethylenic fatty acid totals, respectively, decrease and increase as the iodine values increase. That these are general observations applicable in marine fish oils is shown by the conformity of the high iodine value pilchard oil (Table III), and of medium iodine value oils analysed in this laboratory such as saury (*Cololabis saira*) oil (iodine value of oil 151; Ackman and Jangaard, 1963) and cod liver oil (iodine value of oil 155; Ackman and Burgher, 1964). In considering a relationship between these three basic types of acids it was observed that a more uniform gradation of proportions could be obtained by combining the saturated and monounsaturated fatty acid percentages than by considering them separately (Table III). The balance then gave the percentages of polyunsaturates for the Atlantic herring oils which were plotted against the original oil iodine values and against the calculated ester iodine values (Fig. 1 and 2). Empirical straight lines were drawn through these points and derived formulas tested for accomodation of the iodine value–percentage polyunsaturate value for the high iodine value pilchard oil. It was found that the equations for suitable lines were, respectively:

for oils, per cent polyunsaturates $= 10.7 + 0.337$ (IV oil — 100),

for esters, per cent polyunsaturates $= 13.3 + 0.317$ (calc IV esters — 100).

In the present analyses the scatter of points was slightly less in the oil iodine values (Fig. 1) than in the ester values (Fig. 2). Although both lines were selected to accomodate the pilchard oil of high iodine value the error for the lowest iodine value available (Sample No. 1) is less with the calculated ester iodine value. These equations have general applicability with fish depot fats, and to some degree with flesh phospholipids, and are also reasonably satisfactory with marine animal depot fats (Ackman, 1965). The basis for these relationships is the gradual replacement, for increasing iodine values, of monoethylenic fatty acids (chiefly C_{20} and C_{22}) with polyunsaturated fatty acids of high iodine value (chiefly 20:5ω3, 22:5ω3, 22:6ω3). It can be demonstrated with model calculations that this is the case and that these relationships are inapplicable to terrestrial animal lipids where there are normally higher amounts of acids of lower degrees of unsaturation such as 18:2ω3, etc.

The data of Dunn and Robson (1965) are of interest since these authors did not use gas–liquid chromatography but instead a thin-layer procedure based on silver nitrate-impregnated silica gel, with gravimetric determination of the fatty acid groups. Their replicate analyses (Table III) of one herring oil (presumably European) show higher levels of saturated acids (of the same order as that of Lambertson and Braekkan, 1965), about the same proportion of monoethylenic acids, and consequently lower levels of polyethylenic fatty acids. The detailed information on the polyunsaturated acids is restricted to the dienoic, where the levels are appreciably higher than found in the present survey and in the data of Klenk and Eberhagen (1962).

SATURATED ACIDS

In the other comparative data (Table III) the relationship of 16:0 as per cent of the total saturated acids is nearly constant at 60%. A detailed study (Ackman and Sipos, 1965) of the isolated saturated acids gave similar values of 65% for depot fats in squid, cod, and herring. The herring sample (solvent-extracted body oil only) in question was identical with the No. 4 sample in this study, in which the proportion from the present data is 63.9%. It must, however, be noted that the detailed analysis included the branched chain fatty acids and that the elimination of unsaturated materials permitted more accurate quantitation. The figure of 60% may apply to the majority of the Atlantic herring oils studied, and the values for the Pacific and European herring oils are in general similar. This particular fatty acid (16:0) is evidently a key metabolite in fish and levels are relatively independent of diet (Brenner et al., 1963).

SATURATED AND MONOUNSATURATED ACIDS

The ratio 16:0/16:1 has been suggested (Lewis, 1962) as an indicator of environmental temperature with marine life. The analyses discussed by Lewis were incomplete (see Rodegker and Nevenzel, 1964) but sufficient for this purpose. The attempt to correlate different species was only successful in the broadest terms. In the present study this ratio includes figures 35% higher and lower than the average (1.4), but the Pacific and European herring analyses are generally higher. The temperature generalization does not seem applicable without definite water temperature data and other factors probably influence the ratio in question. With other Pacific species the values were 1.8 (pilchard), 1.9 (Peruvian anchovy, not tabulated, Klenk and Eberhagen, 1962), and 1.8 (Peruvian anchovy, not tabulated, Lambertson and Braekkan, 1965). Lovern (1938) has pointed out that the mean unsaturation of the C_{16} chain length is highest in feeding fish and maximal with the fattest fish, but polyunsaturated fatty acids could influence this value.

TABLE III. Comparisons of properties of interest for some Atlantic h

Property compared	Atlantic herring oils									
	Averages No. 1–12 only	Sample No.								
		1	2	3	4	5	6	7	8	9
Weight per cent saturated	20.0	16.4	19.6	21.7	16.5	20.1	21.7	22.1	20.6	17.8
Weight per cent monounsaturated	60.0	71.5	63.0	58.2	65.4	60.0	57.9	56.3	57.7	61.7
Total weight per cent saturated + monounsaturated	80.0	87.9	82.6	79.9	81.9	80.1	79.6	78.4	78.3	79.5
Total weight per cent dienoic	2.1	1.4	1.7	2.6	1.5	2.4	3.6	2.5	2.0	1.6
Total weight per cent trienoic	1.3	0.9	1.1	1.5	0.9	1.4	1.4	1.8	1.3	1.0
Total weight per cent tetraenoic	3.3	2.3	3.2	3.4	2.6	3.8	2.6	4.0	3.1	3.5
Total weight per cent pentaenoic	8.6	5.3	6.8	7.3	9.5	7.9	7.4	6.9	10.1	9.7
Total weight per cent hexaenoic	4.7	2.1	4.9	5.2	3.7	4.7	5.4	6.3	5.0	4.2
Total weight per cent polyunsaturated	20.0	12.0	17.7	20.1	18.1	20.2	20.4	21.6	21.6	20.0
Hexadecanoic as per cent of saturated	59.5	61.6	60.5	54.7	62.9	57.6	59.0	55.3	61.8	61.0
Ratio 16:0/16:1	1.4	1.0	1.3	1.9	0.9	1.5	1.8	1.9	1.5	0.9
Ratio 16:0/(16:1 + 18:1)	0.56	0.52	0.53	0.68	0.49	0.58	0.45	0.67	0.59	0.4
Ratio 16:1/18:1	0.8	1.1	0.7	0.6	1.1	0.7	0.3	0.5	0.7	0.9
Ratio $(20{:}x\omega6 + 22{:}x\omega6)/(18{:}2\omega6 + 18{:}3\omega6)$	0.82	1.07	0.83	0.58	0.73	0.68	0.46	0.71	0.70	1.2
Ratio $20{:}x\omega3/18{:}x\omega3$	3.3	2.6	2.6	1.9	6.6	2.2	3.1	1.6	4.0	4.3
Ratio $22{:}x\omega3/18{:}x\omega3$	2.4	1.6	2.3	1.8	3.1	1.8	3.5	1.9	2.5	2.8
Ratio $22{:}x\omega3/20{:}x\omega3$	0.78	0.63	0.89	0.95	0.48	0.82	1.12	1.15	0.63	0.6
Ratio $\Sigma\omega3/\Sigma\omega6$	7.5	6.3	7.2	6.8	11.2	6.3	3.3	6.3	7.4	7.7
Ratio $\Sigma n{:}x\omega3/\Sigma n{:}x{-}1\omega3$	7.4	5.8	8.7	7.0	12.4	6.3	4.1	6.1	8.1	7.3

[a] From alkali isomerization data.
[b] European origin assumed.

e Atlantic herring oils, for some other herring oils, and for pilchard oil.

					Pacific herring oils		European herring oils							Pacific pilchard oil
									Dunn and Robson (1965)[b]					
									Average	Replicate analyses				
8	9	10	11	12	(This study)	(Gruger et al., 1964)	Lambertson and Braekkan (1965)	Klenk and Eberhagen (1962)	No. 1–4	1	2	3	4	(Ackman and Sipos, 1964b)
20.6	17.8	17.1	24.4	22.1	24.7	29.0	26.1	20	25.9	24.2	27.1	26.4	25.7	28.8
57.7	61.7	61.9	52.1	54.1	54.6	46.2	50.4	59	58.7	60.2	58.6	58.1	57.9	28.3
78.3	79.5	79.0	76.5	76.2	79.3	75.2	76.5	79	85.6	84.4	85.7	84.5	83.6	57.1
2.0	1.6	1.6	2.0	1.9	1.5	...	...	1.8[a]	3.6	2.5	4.7	4.0	3.1	2.6
1.3	1.0	1.1	1.6	1.5	1.0	...	...	2.0[a]	11.8	13.1	9.4	11.5	13.3	2.0
3.1	3.5	3.4	4.0	4.3	3.1	...	...	4.2[a]						4.9
10.1	9.7	10.3	10.7	10.7	10.0	...	...	4.6[a]						19.4
5.0	4.2	4.5	5.1	5.5	5.0	...	...	5.2[a]						12.9
21.6	20.0	20.9	23.4	23.8	20.6	24.8	23.5	21	15.4	15.6	14.1	15.5	16.4	41.8
61.8	61.0	60.0	61.4	56.6	67.4	63.1	57	65	...	...	...	...	...	56.3
1.5	0.9	0.9	1.7	1.5	2.2	2.2	1.6	2.2	...	...	...	...	...	1.8
0.59	0.44	0.43	0.66	0.68	0.55	0.73	0.70	0.46	...	...	...	...	...	0.79
0.7	0.9	0.9	0.7	0.9	0.3	0.5	0.7	0.3	...	...	...	...	...	0.8
0.70	1.24	1.61	0.64	0.57	1.31	...	...	...	...	...	...	...	...	1.15
4.0	4.3	4.8	3.2	3.0	4.2	...	...	...	...	...	...	...	...	6.0
2.5	2.8	3.1	1.9	2.0	2.7	...	...	...	...	...	...	...	...	5.2
0.63	0.64	0.65	0.60	0.66	0.64	...	...	...	...	...	...	...	...	0.87
7.4	7.7	9.2	8.6	9.9	11.2	...	...	...	...	...	...	...	...	12.7
8.1	7.3	7.5	7.5	7.7	8.2	...	...	...	...	...	...	...	...	6.4

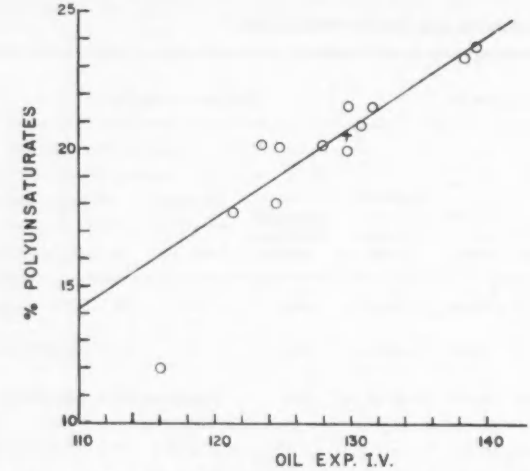

FIG. 1. Plot of per cent polyunsaturated fatty acids indicated by gas–liquid chromatography against herring oil iodine values (Wijs). All Atlantic herring oils (o) except one Pacific herring oil denoted (+).

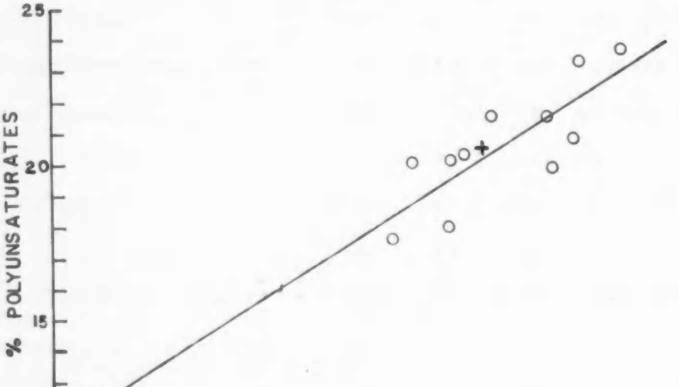

FIG. 2. Plot of per cent polyunsaturated fatty acids indicated by gas–liquid chromatography against iodine values calculated from the composition indicated by gas–liquid chromatography. All Atlantic herring oils (o) except one Pacific herring oil denoted +.

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The ratio 16:1/18:1 (Table III) varies widely, but in general the deviations from the mean are arithmetically opposite to those of the ratio 16:0/16:1. It is possible that in depot fats these two monounsaturated fatty acids are partly interchangeable. The ratio 16:0/(16:1 + 18:1) was therefore investigated (Table III) with better results. With the Atlantic herring oils the maximum deviations from the average are only about 20% (average deviation = 0.08 or 15% as against average deviation of 0.39 or 28% for the 16:0/16:1 ratio). On the basis of the ratio including both monounsaturated fatty acids, the Pacific herring oil analysed in the present study gave a figure the same as the Atlantic average, but the other Pacific analyses were higher (herring, 0.73; pilchard, 0.79; anchovy, 0.79 and 0.76) while the European values were, respectively, lower (0.46) and higher (0.70). Finally a further ratio (not tabulated) was investigated based on (14:0 + 16:0 + 18:0)/(16:1 + 18:1), with an average value of 0.917 but no improvement in precision. These particular investigations suggest that there may be regional peculiarities in the proportions of some of these fatty acids but further data are needed to confirm this, both to establish typical values for Pacific and European herring, and also to include other species with characteristic body depot fats.

Lambertson and Braekkan (1965) have discussed the significance of the 20:1 and 22:1 acids in clupeids. The ratios for 22.1/20.1 (not tabulated) gave an average of 1.44 but of the twelve analyses six fell within the range 1.43–1.54, and four within the range 1.23–1.30. The two other results were, respectively, higher and lower than these ranges. Lambertson and Braekkan (1965) in their own work on one Norwegian herring oil found a ratio of 1.57.

POLYUNSATURATED ACIDS

The metabolic pathways affecting polyunsaturated fatty acids are apparently the same in marine life as in mammals (Kayama, 1964; Kayama et al., 1963). Phytoplankton produce the higher polyunsaturated fatty acids characteristic of fish oil (Ackman et al., 1964; Kayama, 1964) as well as shorter chain fatty acids of interest such as 18:2ω6, 18:3ω6, and 18:3ω3. The latter are, however, minor components of fish oils as the intermediate zooplankton convert them into the C_{20} and C_{22} highly unsaturated fatty acids (Kayama, 1964). Fish can carry out the same synthesis if necessary (Brenner et al., 1963; Kayama et al., 1963; Ackman, 1964). A number of ratios of particular fatty acids were investigated (Table III) but since several of these are based on minor components the order of accuracy in these instances is not very high. In the linoleic type (ω6) acids the ratio (20:xω6 + 22:xω6)/(18:2ω6 + 18:3ω6) is moderately consistent. The variations in this ratio may be partly associated with variations in 18:2ω6, and since the oils included stomach contents some food organisms rich in this acid may be involved. If this particular ratio is susceptible to influence by diet, the Pacific herring oil may also be affected, although in comparisons of this and other ratios the validity of taking only one analysis as typical is doubtful.

In the linolenic ($\omega3$) type acids the ratio $20{:}x\omega3/18{:}x\omega3$ increases with increasing iodine value, although this is less definite in the ratio $22{:}x\omega3/18{:}x\omega3$. This could account for the higher figures obtained with the high iodine value Pacific pilchard oil. The proportions of fatty acids in the ratio $22{:}x\omega3/20{:}x\omega3$ include the polyunsaturated major components in both chain lengths and the ratios are therefore based on more accurate data. There are wide variations in the ratio but a definite decrease with increasing iodine value. This is not observed in the pilchard oil but there must be limitations on the spread of values for this ratio. The overall ratio $\Sigma\omega3/\Sigma\omega6$ does increase with increasing iodine value and this is very marked in the pilchard oil. This ratio may have significant effects in studies on the hypercholestemic effects of marine oils (Peifer et al., 1965). Finally, within the linolenic system the ratios of total final products of maximum unsaturation were compared with the potential immediate precursor of the same chain length lacking the one double bond closer to the carboxyl group. This ratio $\Sigma n{:}x\omega3/\Sigma n{:}x{-}1\omega3$, with the exception of one very high and one very low value (No. 4 and 6) is moderately consistent not only in the Atlantic and Pacific herring oils but in the pilchard oil as well.

CONCLUSIONS

Marine oils possess typical compositions based on a more or less constant proportion of saturated fatty acids and varying proportions of either low iodine value monoethylenic fatty acids or very high iodine value polyunsaturated fatty acids. It is possible to correlate the proportion of polyunsaturated fatty acids as indicated by gas–liquid chromatography with the iodine value of the oil through a simple mathematical relationship independent of species.

Individual fatty acid components vary widely in analyses of oils from the same species, and the proportions by chain length also vary widely, with only limited correlation of these values and iodine value. In Atlantic herring oils the level of 16:0 fatty acid may vary somewhat but averages 60% of the total saturated fatty acids. The most consistent relationship of 16:0 to monounsaturated fatty acids is with the sum of 16:1 and 18:1 rather than with 16:1 alone. Relationships among the polyunsaturated fatty acids are such that the proportion of C_{18} linoleic type acids to C_{20} and C_{22} successor acids does not appear to vary systematically. The proportions of C_{20} and C_{22} linolenic-type acids increase in relation to the C_{18} linolenic types as iodine value increases, but the C_{22} linolenic-type acids decrease in relation to the C_{20} linolenic-type acids as iodine value increases. The total of linolenic-type acids in proportion to the total linoleic-type acids increases with increasing iodine value. The proportion of linolenic-type acids compared to immediate precursors of the same chain length is apparently independent of iodine value. The applicability of some of these relationships to other herring oils and some body oils of similar species suggests that there may be similarities by species with some ratios, by region of origin with other ratios, and some relationships reasonably independent of species or origin.

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Responses to Light of Young Atlantic Salmon (*Salmo salar*) and Brook Trout (*Salvelinus fontinalis*)[1,2]

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ABSTRACT

Responses of young Atlantic salmon (*Salmo salar* L.) and of brook trout (*Salvelinus fontinalis* (Mitchill)) to different levels of illumination and to overhanging and submerged cover were compared in the laboratory. At surface illuminations between 0.4 and 160 ft-c trout were either slightly photopositive or showed no preference for light or dark areas, while salmon were photopositive. At illuminations above 300 ft-c both species were photonegative when the light area had a smooth, unbroken substrate. Under these conditions starvation induced salmon into the light, but trout remained photonegative, although slightly less so than well-fed trout. With submerged cover (plants, broken flowerpots, etc.) in the light area, salmon were strongly photopositive, while trout were photonegative. Salmon were less photopositive, but still preferred the light area, with submerged cover in both dark and light areas. In most experiments trout maintained position at the edge of the shadow caused by overhead cover, looking out into the light area from the dark.

The significance of these results to habitat segregation by the two species where they are sympatric in nature is discussed.

INTRODUCTION

BROOK TROUT and young Atlantic salmon live together in many rivers of eastern Canada. In some headwater regions they appear to share the stream habitat with no other fish except the American eel (*Anguilla rostrata*). Both trout and salmon spawn in fresh water but their life histories are not identical. Some trout in coastal streams move out to salt water and return later to spawn as "sea trout"; others never go to sea. Young salmon spend 2 or more years in fresh water, migrate to sea as smolts, and then return to spawn 1 or more years later. In fresh water young salmon are found throughout the length of rivers, while trout occur mainly in the cooler headwaters. Where they occur together the two species appear to be segregated, at least during the summer months, by occupying different microhabitats (Gibson, MS, 1965).

Gibson studied the freshwater habitats of both species by underwater swimming in the Northwest Miramichi River, New Brunswick, during the summers of 1963 and 1964. He observed that brook trout tend to stay under cover, in pools, in relatively slow-moving water during the day, moving out into the open to feed during evening and early morning. He found young

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salmon typically in unshaded, shallow, fast-flowing riffle areas during the day. Gibson suggested that the two species shared the stream habitat by differential responses to cover and illumination, water velocity, and possibly temperature. He found little evidence of interspecific territorial aggression such as that observed between salmonid fishes by Kalleberg (1958), Lindroth (1955), and Newman (1956).

Pinhorn and Andrews (1963, 1965) have suggested, on the basis of laboratory experiments, that young Atlantic salmon are photonegative. This is contrary to evidence from field studies (e. g. Gibson, MS, 1965; Keenleyside, 1962) which showed that young salmon in fresh water generally spend daylight hours in shallow, unshaded areas.

The experiments described in this paper, therefore, had two objectives: first, to study the behavior of young Atlantic salmon under various conditions of illumination, to see if they can be classed as photonegative or photopositive; second, to compare the responses of salmon and trout to different conditions of light and shade, to assess the importance of photic responses in segregating the two species under natural conditions.

MATERIALS AND METHODS

Experiments were conducted at the University of Western Ontario between November 1964 and May 1965. Appendix I lists the dates, temperatures, and the number and sizes of fish used in each experiment. Underyearling Atlantic salmon were obtained from the Department of Fisheries of Canada, Fish Culture Station, Saint John, N.B. They had hatched on April 30, 1964. The parents were of fall-run stock (i.e. entered fresh water to spawn in the fall of 1963) from the Northwest Miramichi River, N.B. Brook trout were supplied by the Chatsworth Fish Hatchery, Ontario, where they had hatched in early December 1964. The parent trout were progeny of a "domestic" stock (hatchery-held for several generations) crossed with wild trout from Lake Nipigon in October 1958.

The trout and salmon were maintained separately in two large holding tanks at a constant temperature of 9 C. The tanks were well aerated and supplied with a small flow of tap water, filtered through glass wool and charcoal. Each tank was illuminated with a 25-w incandescent bulb and the fish were exposed to a daily 12-hr photoperiod between 8 AM and 8 PM. The fish were fed daily, in all regions of each tank, on chopped beef liver and heart, trout food pellets, frozen brine shrimp, or mosquito larvae.

Experiments were conducted in a separate room, where temperatures were not rigidly controlled (see Appendix I). Fish were held in 150-litre acclimation aquaria in the experimental room for 1 week before being tested. Each of these aquaria was illuminated by a 25-w incandescent bulb, planted to provide shade and cover, and well aerated. Fish in acclimation tanks were given a 12-hr photoperiod between 8 AM and 8 PM, and were fed as in the holding tanks.

Two identical experimental aquaria were used. These were of black-painted plywood with a glass front, 137 cm long, 61 cm wide, and 46 cm deep. The bottom of each was covered with sand about 4 cm deep. Each tank was divided into 18 equal "compartments" between sand and water surface, by five vertical and two horizontal lines drawn on the glass front and on the wooden back. The tanks were full in experiment 1, but to increase uniformity of illumination in the water in all other experiments they were kept two-thirds full and only the lower 12 "compartments" were used. Filters in the rear corners cleaned and circulated the water. Standing water was used throughout.

Each tank had a horizontal black wooden cover over half the surface. A black wooden flange from the cover dipped 2.5 cm below the water surface in experiment 1, and for the remaining experiments, a black plastic flange extended 4 cm below the water surface. The cover and flange divided each tank into a light and dark area. Each tank was illuminated by a fluorescent tube (General Electric F40 Cool White) 117 cm long, supported in a shade 17.5 cm above the tank. In all but experiment 1, this was supplemented by four 150-w Sylvania Reflector flood lamps, placed 30.5 cm above the water, near the four corners of the light area. The light and dark areas were alternated in some experiments by reversing the positions of flood lamps and cover. Light intensities in the aquaria were controlled with a "powerstat" (Superior Electric, type 3PN 116). For all experiments a black cloth screen, with observation slit, was supported about 25 cm away from the front of each tank. In experiment 1 another black cloth covered the apparatus and observer, but in all other experiments this was made unnecessary by blacking out the entire experimental area with curtains.

Light intensity was measured with a Photovolt Universal Photometer, model 200. The photocell was encased in a waterproof box, made of 0.85-mm thick plastic, weighted at the bottom. Light intensities were measured by placing the photocell in a horizontal position in the middle of the front, centre, and back of each compartment. Readings were taken in both aquaria, with covers and screens in place. The only recorded differences in illumination between the two tanks were caused by slight variations in placing the photocell in each compartment. Underwater readings were multiplied by a factor of 1.054 to correct for the waterproof case. To prevent water temperatures from rising under the flood lamps, three glass tubes, 123 cm long, 15 mm diameter, were partly buried in the sand in each tank. The tubes were joined by rubber tubing and cold tap water was run through them. After mid April, with rising room temperatures, the water was first run through copper tubing in an ice-brine bath.

Five separate experiments were carried out. Ten salmon and 10 trout were used in each of experiments 1, 2, and 4. In experiment 3, 13 different fish of each species were used, as described below. Ten salmon were used in experiment 5. Some of the fish were used in more than one experiment, but except for experiment 3, none was used twice in the same experiment. In all experiments fish were tested singly. One fish was placed in each aquarium in the early evening and had at least 12 hr continual darkness before a test began. A test consisted of several 10-min observation periods during 1 day. Differences in procedure between experiments are described below.

Response to light was measured by recording the compartment the fish was in at regular intervals for 10 min. Records were made at 5-sec intervals in experiment 1 for a total of 120 recordings, and for convenience in tabulating, at 6-sec intervals in experiments 2–5 for a total of 100 recordings per observation period per fish. If a fish was on a compartment boundary, it was recorded in that in which it had its head. Seconds were counted with a metronome, and positions were recorded by dropping plastic rings on a peg-board, the pegs corresponding to the aquarium compartments. Thus, the observer could watch the fish continuously. At the end of the 10-min period the number of position records for compartments in the uncovered area and in the covered area were summed separately, to give the total number for light and dark.

At the end of a day's test, each fish was anaesthetised in M.S. 222, weighed, measured, marked by fin clipping, and returned to its holding tank. Fish were well fed the day before a test, except for part of experiment 3, but were not fed in the experimental tanks.

EXPERIMENT 1

Fish were exposed to four different light intensities which gave surface illuminations, measured in the centre of the light area, of 0.4, 58, 110, and 160 ft-c. Appendix II shows the range in illumination throughout the aquaria for this experiment, when fluorescent tubes alone were used.

Lights went on at the highest intensity at 8 AM. A 10-min observation period was made at 9 AM, the illumination was then decreased to the next lower level, and after 1 hr another 10-min observation period was made. In this way the illumination was changed after each observation period to the next lower intensity, until the lowest was reached at 11:30 AM. Two observation periods were made at this intensity, then illumination was raised after each period to the next higher intensity, the highest being reached at 5:10 PM. There were thus eight observation periods in experiment 1. The fish were exposed to each light intensity for 1 hr before their positions were recorded. Immediately after the 12:30 PM period the tank covers were interchanged, alternating the dark and light experimental areas.

EXPERIMENT 2

This experiment tested the fishes' reactions to changing illumination under brighter light, beginning with a low illumination, increasing to the highest, and then decreasing again. Three light intensities, A, B, and C were used. Surface illumination in the light area varied from 164 to 410 ft-c (A), 197 to 756 ft-c (B), and 340 to 1220 ft-c (C). Illumination throughout the aquaria under these intensities is shown in Appendix III.

Fish were exposed to different illuminations in the following order: A, B, C, C, B, A. Lights went on at intensity A at 8 AM and at 9 AM a 10-min observation was carried out, then the illumination was changed to B, and at 10:10 AM a second observation was made, and so on. Covers were reversed after the 11:20 AM recording.

EXPERIMENT 3

This experiment tested the effect of starvation on light responses by the two species. Constant high light intensity (C) was used. The fish were not disturbed by alternating dark and light areas in the middle of a test; instead the covers were reversed every second day. Lights went on at 8 AM, and six 10-min observation periods were made at 2-hr intervals, the first at 9 AM, the last at 7 PM. Fed fish had been well-fed the day before testing; starved fish had been without food for 5–9 days.

The experiment was done in two periods; between March 7 and 23, and between April 16 and 26 (Appendix I). With each species five tests were done with fed fish and five with starved fish in each of the two experimental periods. Two fish in the first period were used in both fed- and starved-fish tests. In the second period the same five fish were used for both fed- and starved-fish tests. Thus, 13 different animals of each species were used in 20 tests in experiment 3.

EXPERIMENT 4

This experiment was done to test the effect of underwater cover on the light reactions of Atlantic salmon and brook trout. As in experiment 3, six observation periods were made in each test, at 2-hr intervals between 9 AM and 7 PM, at a constant high intensity (C). Aquarium covers were reversed every second day.

Submerged cover was in the light area only, in the form of twigs, plants, and two pieces of broken flowerpot. One flowerpot piece was 21.5 by 21.5 cm; the other was 7.5 by 7.5 cm. The plants were a mixed bunch of *Myriophyllum*, *Elodea*, *Vallisneria*, and *Cabomba*, about 23 cm in diameter. The twigs were from alder and willow trees in a bunch about 20 cm in diameter. Plants and twigs reached from the tank bottom to the water surface. Cover was similar in both experimental aquaria.

EXPERIMENT 5

In this experiment underwater cover was provided in both dark and light areas, and only salmon were tested. The submerged cover in each area was the same as that used in experiment 4, but in one aquarium hollow bricks were substituted for flowerpots, one brick in the dark and one in the light. Each brick had a partition forming two cavities, and was 12.5 cm high, 15 cm wide, and 30.5 cm long. As in experiments 3 and 4, observations were made at a constant light intensity (C) at 2-hr intervals, between 9 AM and 7 PM, for a total of six observation periods. Covers were reversed every second day.

Illumination among submerged cover in the light area was similar in experiments 4 and 5. Flowerpots and bricks created dark areas (<5 ft-c), but light intensities among plants and twigs varied from 150 to 380 ft-c, i.e. they provided only slight shade. Because of the low light intensity in the flowerpots, fish in a flowerpot in the light area were recorded as being in the dark. Fish among twigs or plants in the light area were recorded in the light.

Significance of the difference in occurrence of fish in light and dark areas was assessed in all experiments by chi-square, with $P < 0.05$ taken as showing significance.

RESULTS

EXPERIMENT 1

Under the relatively low illuminations of this experiment salmon were predominantly photopositive, and trout were usually less photopositive, or showed no preference for either half of the apparatus (Fig. 1). Salmon were significantly photopositive at the $P<0.05$ level at 4 PM, and at the $P<0.01$ level at all other times, except at 12:30 PM when they were significantly photonegative ($P<0.05$). Trout were significantly photopositive ($P<0.01$) at 11:20 AM and at 1:40, 2:50, and 5:10 PM. At 12:30 PM trout were significantly photonegative ($P<0.01$), and at other times they showed no light preference. The difference in response of the two species was significant ($P<0.01$) at intensities

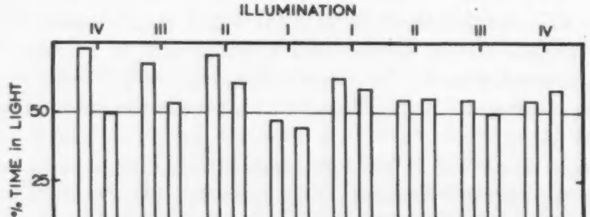

FIG. 1. *Experiment 1.* Effect of changing illumination on distribution of salmon (S) and trout (T) in aquaria. Numbers I–IV represent surface illuminations in centre of light area of 0.4, 58, 110, and 160 ft-c, respectively.

IV, III, and II in the morning, and at the $P<0.05$ level at intensities I and III in the afternoon. In all these cases salmon were more in the light than trout. At other times the responses of the two species were not significantly different.

EXPERIMENT 2

Under the higher illumination of this experiment salmon and trout were significantly photonegative ($P<0.01$) at all light intensities (Fig. 2). At 9 AM and at 10:10 AM salmon were more in the light than trout ($P<0.01$), and at 1:40 PM and 2:50 PM ($P<0.05$), and at 12:30 PM ($P<0.01$) trout were more in the light than salmon. At 11:20 AM there was no significant difference in the light reactions of the two species.

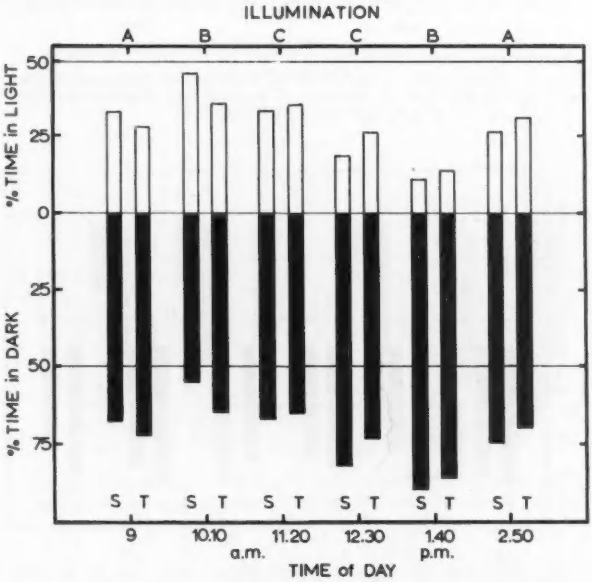

FIG. 2. *Experiment 2.* Effect of higher levels of changing illumination on distribution of salmon (S) and trout (T) in aquaria. A, B, and C represent low, medium, and high levels of illumination, respectively. See text for surface illuminations in light area.

EXPERIMENT 3

Results of tests from March 7 to 23 were similar to those from April 16 to 26, so data from the two series were combined (Fig. 3 and 4). Fed salmon were photonegative at all times ($P<0.01$) except at 11 AM, when they showed no significant preference for light or dark. Starved salmon were photonegative at 9 AM and 11 AM ($P<0.01$), showed no preference at 1 PM, and were photopositive at 3, 5, and 7 PM ($P<0.01$). There was a significant difference ($P<0.01$)

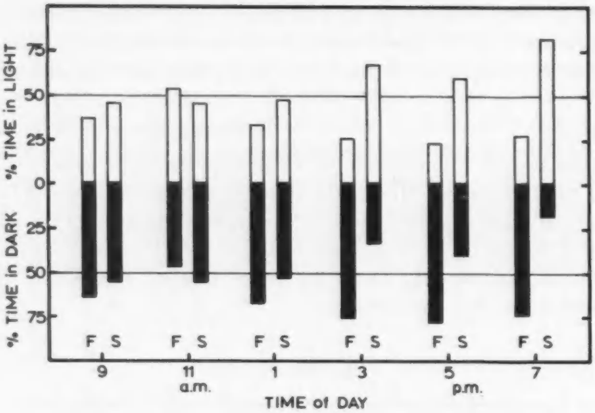

FIG. 3. *Experiment 3.* Influence of starving on responses of salmon to constant light. F, fed fish; S, starved fish. Surface illumination in light area 340–1220 ft-c.

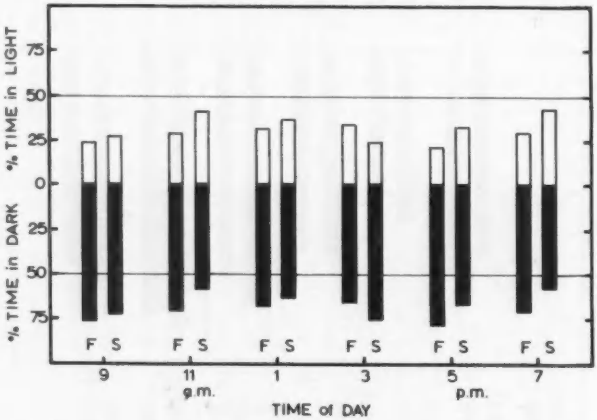

FIG. 4. *Experiment 3.* Influence of starving on responses of trout to constant light. F, fed fish; S, starved fish. Surface illumination in light area 340–1220 ft-c.

at all times between starved and fed salmon. At 11 AM starved fish were less in the light than fed fish, but at all other times starved fish were more in the light than fed fish (Fig. 3).

Both fed and starved trout were photonegative ($P<0.01$) all day (Fig. 4). At 11 AM, 5 PM, and 7 PM ($P<0.01$) and at 1 PM ($P<0.05$) starved trout were more often in the light than fed trout. At 3 PM they were more often in the dark than fed trout ($P<0.01$), and at 9 AM there was no significant difference between them.

EXPERIMENT 4

The conditions of this experiment produced a marked difference in response to light between trout and salmon (Fig. 5). Salmon were consistently photopositive and trout photonegative ($P<0.01$ in both cases). Over the whole day, the salmon spent 75% of the time in the light, while the trout spent only 24% of the time in the light. In other words, salmon responded to the submerged cover in the light area by staying in the light much of the time, while the trout behaved as they had in previous experiments at this illumination (Fig. 2 and 4), and remained mostly under the overhanging cover.

In a total of 6000 observations for the whole experiment salmon were recorded 14 times (3 salmon) in the large flowerpot, 98 times (2 salmon) among or close to plants, and 221 times (2 salmon) among or close to twigs. The twigs count was largely influenced by one fish spending the 11 AM observation period on the bottom between the twigs and the side of the tank, and another fish spending the 11 AM period resting among the twigs and some dead grass near the surface.

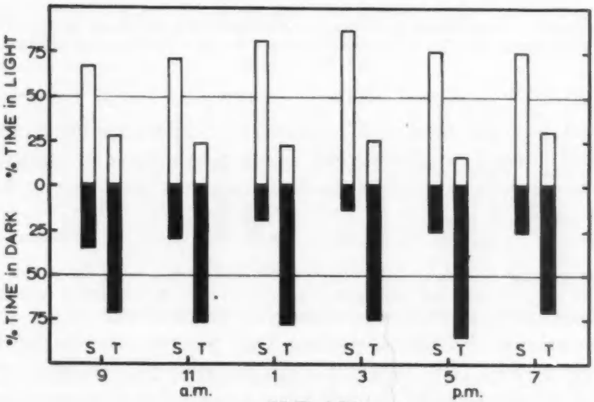

FIG. 5. *Experiment 4.* Effect of submerged cover in light half of aquaria on light responses of salmon (S) and trout (T). Surface illumination in light area 340–1220 ft-c.

Five trout were recorded for 47 observations in the large flowerpot. These were mainly brief, apparently exploratory excursions, but one trout at 11 AM spent 18 counts, and another at 7 PM spent 16 counts, in the large pot. In both cases the fish faced the light from inside the pot. Two trout were recorded for one count each in the plants, and none among the twigs.

EXPERIMENT 5

Only salmon were tested in this experiment and they were not as strongly photopositive as in experiment 4 (Fig. 6). At 11 AM they were photonegative

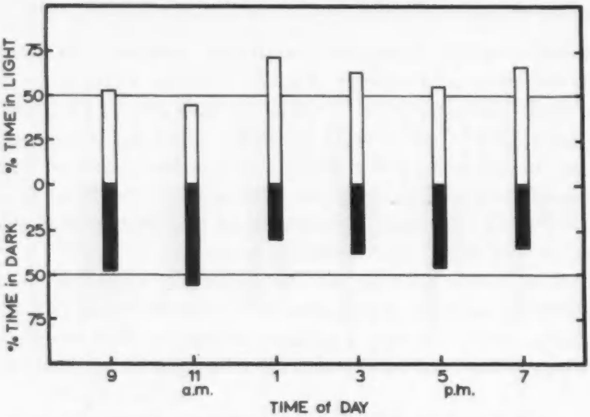

FIG. 6. *Experiment 5*. Effect of submerged cover in both dark and light areas on light responses of salmon. Surface illumination in light area 340–1220 ft-c.

($P < 0.01$) and at 9 AM there was no significant difference in response to light or dark. At all other times the salmon were photopositive ($P < 0.01$). They did not swim into submerged cover in the dark area, but the cover in the light area was used occasionally.

GENERAL RESULTS

In all experiments trout, and especially salmon, were more active in the morning than later in the day. Sometimes they tried to jump out of the aquaria, a few trout succeeding. Tests in which trout jumped out were discarded. During this period of greater activity the fish often spent much time swimming up and down near the front glass. Although activity was not recorded, the higher proportion of observations in the surface compartments during the morning than in the afternoon indicates the greater activity of both species at the former time (Fig. 7). When quiet the fish usually stayed close to the bottom. Hoar et al. (1957) described similar active swimming by the aquarium walls in sockeye salmon smolts as "escape behavior." Pinhorn and Andrews (1965) interpret similar behavior by Atlantic salmon as "positive thigmotaxis." In our experiments this behavior usually disappeared in the afternoon observation periods, suggesting it is escape or exploratory behavior. The fish appear to "become accustomed to" the aquarium conditions after an initial period of heightened activity.

At all light intensities, and particularly at the higher ones, trout commonly were positioned in the dark area at the edge of shadow from the overhead cover, looking out towards the light. They rested horizontally, on or close to the bottom, and made occasional forays into the light area. Similar behavior by brook trout in apparatus providing light and dark areas was reported by

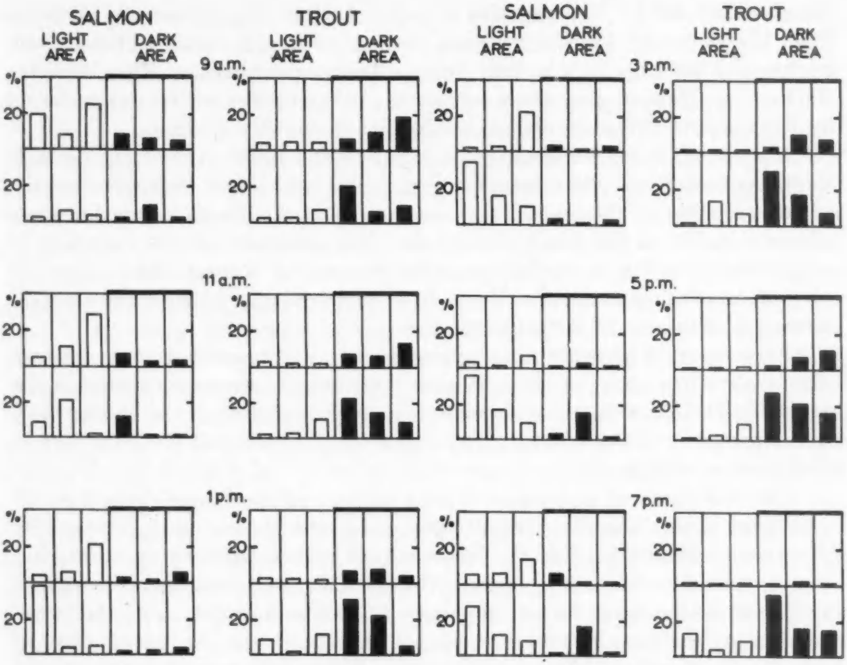

FIG. 7. Distribution of trout and salmon in compartments of experimental aquaria in experiment 4, at different times of day. Solid black bars represent per cent time in dark area. Open bars represent per cent time in light area.

Sullivan and Fisher (1954). This tendency shows up as a high percentage of time spent in the dark compartment closest to the light. Figure 7 illustrates the different responses of salmon and trout to the edge of shadow in experiment 4. Occasionally a trout had its head in the light, and the rest of its body in the dark, in which case it was counted in the light. Starved trout commonly did this in experiment 3.

Salmon did not rest near the edge of the shadow as consistently as trout, and when they did, they faced in any direction, not only into the light. In both light and dark areas salmon frequently held position near the bottom with the body tilted head-up, sometimes as much as 45° from the horizontal.

DISCUSSION

Experiments 1 and 2 show the effects of two different ranges of illumination on salmon and trout behavior. Under the relatively low illuminations of experiment 1, both species, and especially salmon, were mainly photopositive, corresponding to field observations that salmon are in the open during daylight hours, and trout often move out into the open as darkness falls in the evening

(Gibson, MS 1965). The negative response to light by salmon at 12:30 PM (Fig. 1) was caused by the tendency of some salmon to settle to the bottom in the dark area during the first hour of lowest illumination. This behavior remains unexplained, since these fish usually became more active and preferred the light area in the second consecutive hour at this illumination.

Under the higher illuminations of experiment 2 both species were strongly photonegative. Trout show a similar response to bright light in nature, but the salmon response in this experiment was unexpected. The tendency for some salmon to settle on the bottom in the dark end was more pronounced than in experiment 1, and may partially explain the results. A more likely cause, as later experiments showed, was the absence of protective cover in the smooth-bottomed, open area of the apparatus.

Experiment 3 showed that hungry salmon will move out into constant bright light after several hours exposure even though protective shelter is not available, whereas well-fed salmon continue to stay mainly in the shade under overhanging cover. Both hungry and well-fed trout preferred the dark area in this experiment (Fig. 4).

The conditions of experiment 4, with submerged cover and shelters in the open area, under constant bright light, produced the greatest interspecific difference in behavior (Fig. 5). Trout stayed under overhanging cover, but salmon moved out into the open, although they were well fed throughout these tests. The salmon did not appear to be hiding directly among the twigs, branches, or shelters, but the presence of these structures in the brightly-lit area clearly stimulated them to stay there. This result suggests that salmon stayed under cover in experiments 2 and 3 (when well fed) because the open area under high illumination was too bare and unsheltered. In nature, young salmon live mainly in open, uncovered riffle areas where shelter of two sorts is available. First, the substrate is typically of pebbles or rocks, among which alarmed young salmon are quick to hide (Keenleyside, 1962) and second, the water surface in riffle areas is turbulent. A human observer has great difficulty in seeing fish in rapids because of the broken turbulent surface and salmon are probably protected from surface predators by this poor visibility. Trout stayed under the overhanging cover, even with submerged debris in the open area (Fig. 5). The open, loose network of branches and the flowerpot pieces did not appear to provide suitable shelter for trout. Results of experiment 4 suggest a basic interspecific difference in habitat preference. Salmon stay in bright light when the immediate surroundings provide structures among which they can hide when frightened. Trout seem to prefer shaded areas from which they may emerge to snatch food, but where they can also retreat into darker corners when alarmed.

Experiment 5 involved salmon only, and with submerged cover in both areas of the apparatus they still tended to stay in the open, although not as consistently as in experiment 4. The less-marked positive response to light may be related to submerged cover providing protection in both light and dark areas in experiment 5 but only in the light area in experiment 4, or it could be related to higher temperatures in experiment 5. In Goldmine Brook, Nova Scotia, Elson (MS, 1942) found young salmon mostly out in the open, exposed to direct sunlight, at water temperatures of 13 C and less. At temperatures above 13 C the fish tended more often to be under cover.

In experiment 5 the average temperature was about 3 C degrees higher than in experiment 4 (Appendix I) but in experiment 5 the average temperature at 9 AM was 16.3 C (range 14–18 C), and at 7 PM was 20.7 C (range 20–23.5 C), and the salmon were least photopositive at 9 AM and 11 AM, when the water was coolest (Fig. 6). The greater tendency to stay under cover in experiment 5 was thus probably due to attraction by submerged cover in the dark area, rather than a temperature-induced change in light response.

In summary, our results showed that young Atlantic salmon were predominantly photopositive. They stayed in the shadow of overhanging cover if adequate shelter was not available in brightly-lit areas, although when hungry they moved out into bright light even without shelter. In our experiments shelter in the form of submerged, loose debris stimulated salmon to stay in the light. Young brook trout, however, were mainly photonegative, especially at higher light intensities. They tended to stay under overhanging cover whether well fed or hungry, and whether submerged debris was available in brightly-lit areas or not. Trout showed a marked tendency to hold position close to the border of the shadow created by cover under which they hid.

Pinhorn and Andrews (1963) found that young Atlantic salmon were photonegative in a horizontal light gradient at all but very low illuminations (0.1 ft-c) using overhead lights, and that when the light source was at one end of the apparatus (Pinhorn and Andrews, 1965) salmon were photonegative at all intensities used. The discrepancy between their results and ours may be due to experimental procedure. In their experiments with overhead lights, observations were begun 30–90 min after fish were placed in the apparatus, and from 10 sec to 30 min after experimental lights were turned on. In our tests fish were in the apparatus for at least 13 hr, and were exposed to the first experimental illumination for 1 hr, before observations began. Also, in all their experiments salmon were tested in groups, whereas we used single fish.

In one series of tests, Pinhorn and Andrews (1965) left their salmon in the apparatus overnight before beginning observations. The following day the fish, although still photonegative, were less photonegative than in other tests. The chief cause of this change in light response was said to be an increased frequency of aggressive territorial behavior in tests where fish had been in the apparatus overnight. Our results suggest that an active movement into brighter light may have been involved.

Our results support Gibson's (MS, 1965) conclusion that different responses to light are partly responsible for the observed habitat segregation of the two species in nature. The effects of light are most pronounced during periods of brightest sunlight, when trout are mainly under cover and salmon are in the open. At dusk trout move more into the open and habitat segregation is less pronounced. The experimental results confirm this relationship; the only case in which trout were photopositive to any degree was experiment 1, in which lowest illuminations were used. In all other experiments trout were predominantly photonegative.

Different light responses are not likely to be solely responsible for habitat segregation. Gibson (MS, 1965) found in the field that young Atlantic salmon and brook trout respond differently to water velocity and to temperature. Also, Hartman (1965) has shown the complexity of interrelationships between closely related sympatric species and their environment. He found that young coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*) tend to segregate into separate microhabitats in spring and summer, when populations are highest and interspecific aggression greatest, even though laboratory experiments showed that responses of the two species to light, cover, water depth, and velocity were most similar at that time. In fall and winter with lower population densities and less aggression, and when their responses to environmental variables in the laboratory were more distinct, the two species tended to share the same stream habitat. This apparent paradox was explained by an earlier hypothesis of Nilsson (1963) that with strong competition for limited resources (e. g. food and space) two closely related species tend to separate into distinct microhabitats by feeding on different prey organisms and by frequent interspecific aggressive encounters. When competition is less pronounced, as in the fall and winter situation, food preferences are similar, aggression is less frequent, and microhabitats largely overlap.

Although the experiments reported in this paper were done between November and May, the fish were exposed in the laboratory to a 12-hr photoperiod and to water temperatures characteristic of salmon and trout streams in summer. Aggressive behaviour was eliminated by testing fish singly. The results strongly suggest that the observed habitat segregation between young Atlantic salmon and brook trout is at least partly a result of interspecific differences in response to light and cover.

ACKNOWLEDGMENTS

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(Appendixes I–III *follow*)

APPENDIX I. Dates of experiments (November 1964 to May 1965), number and size of fish, temperature data. Length and weight data are means, with range in parentheses. Temperatures are means at beginning and end of each day's observations, with range during experiment in parentheses.

		No. used		Standard Length (*cm*)		Weight (*g*)		Temperature (*C*)	
Experiment	Date	Salmon	Trout	Salmon	Trout	Salmon	Trout	Exp. tanks	Acc. tanks
1	Nov. 26–Dec. 2	10	10	6.7 (6.0–7.8)	11.4 (10.5–14.0)	3.9 (2.7–6.0)	21.2 (15.0–42.5)	14.1 (12.2–16.2)	13.5 (12.5–14.5)
2	Feb. 18–Mar. 3	10	10	6.5 (5.5–8.3)	13.8 (11.0–17.0)	3.8 (1.5–7.3)	37.4 (22.5–65.6)	12.3–14.8 (10.0–16.0)	11.4–12.1 (10.0–13.5)
3	Mar. 7–14 (starved)	5	5	6.5 (5.7–7.5)	14.3 (12.0–16.0)	3.6 (2.0–5.8)	40.8 (24.4–53.8)	13.5–16.9 (12.0–19.0)	12.2–12.3 (12.0–13.0)
	Mar. 15–23 (fed)	5	5	6.8 (5.4–9.0)	14.4 (12.0–18.0)	4.5 (1.9–10.0)	44.1 (24.4–80.1)	13.3–16.8 (12.0–18.0)	12.6–12.1 (12.0–13.0)
	Apr. 16–20 (fed)	5	5	7.1 (5.5–8.0)	13.7 (11.5–14.0)	5.1 (2.5–6.7)	38.7 (27.6–41.5)	14.0–17.4 (13.0–19.5)	12.5–13.3 (12.0–14.5)
	Apr. 21–26 (starved)	5	5	7.1 (5.5–8.0)	13.7 (11.5–14.0)	5.1 (2.5–6.7)	38.7 (27.6–41.5)	14.3–18.0 (13.0–20.0)	12.8–12.8 (12.0–13.5)
4	Apr. 2–14	10	10	7.1 (5.5–10.0)	13.2 (10.5–15.0)	5.6 (2.5–14.0)	34.2 (18.3–57.6)	13.6–17.6 (11.0–20.5)	13.7–13.9 (11.5–16.0)
5	Apr. 29–May 3	10	0	6.9 (5.5–8.0)	–	4.8 (2.5–7.1)	–	16.3–20.7 (14.0–23.5)	16.8–18.1 (15.5–19.5)

APPENDIX II. Illumination (ft-c) in the 18 compartments of experimental tanks, experiment 1. Numbers I–IV represent increasing illumination. Figures from top to bottom in each vertical group of three represent light intensity in back, centre, and front of each compartment.

Light area				Dark area		
			I			
0.05	0.05	0.05				
0.10	0.21	0.21		0	0	0
0.05	0.05	0.05				
0.10	0.16	0.10		0		
0.10	0.21	0.21		0.02	0	0
0.10	0.16	0.10		0		
0.10	0.21	0.21		0.02		
0.10	0.21	0.21		0.02	0	0
0.10	0.21	0.21		0.02		
			II			
5.5	7.6	9.0		0.05	0.10	0.07
25.2	43.2	47.5		0.10	0.10	0.10
5.1	10.0	12.6		0.85	0.10	0.06
10.0	25.3	16.5		0.58	0.05	0.05
22.1	34.7	28.4		2.95	0.05	0.05
13.5	25.3	21.8		0.63	0.08	0.05
12.6	21.1	20.0		3.58	0.08	0.05
16.9	27.4	25.2		3.68	0.10	0.05
16.6	27.4	24.2		2.84	0.10	0.05
			III			
11.4	16.9	19.5		0.15	0.21	0.16
50.3	99.0	103.0		0.16	0.21	0.16
10.9	22.6	26.3		0.16	0.21	0.16
20.0	48.5	33.6		1.21	0.10	0.10
41.2	70.7	70.7		7.15	0.10	0.13
25.3	47.5	46.4		1.42	0.16	0.10
25.3	38.0	35.8		7.40	0.16	0.09
30.6	54.9	49.6		7.90	0.21	0.10
30.6	52.6	48.5		6.32	0.21	0.10
			IV			
16.6	24.7	26.3		0.21	0.32	0.21
78.0	137.0	146.7		0.21	0.32	0.26
15.8	30.5	37.8		0.23	0.32	0.21
25.2	69.5	50.6		1.80	0.16	0.16
60.0	101.0	101.0		10.54	0.21	0.21
34.8	67.3	67.3		2.10	0.21	0.21
31.6	56.0	50.3		10.50	0.21	0.10
45.4	82.2	71.7		11.60	0.32	0.16
44.3	76.0	70.5		8.95	0.32	0.16

APPENDIX III. Illumination (ft-c) in the 12 compartments of experimental tanks, experiment 2. A, B, and C represent increasing illumination. Figures from top to bottom in each vertical group of three represent light intensity in back, centre, and front of each compartment.

Light area				Dark area		
			A			
284	216	310		0.52	0.89	0.74
189	216	262		0.68	1.05	0.95
310	216	302		0.89	1.26	1.05
198	189	232		2.53	0.42	0.53
172	172	216		4.85	0.53	0.63
262	232	262		3.80	0.68	0.74
			B			
430	353	483		0.95	1.47	1.21
318	345	380		1.05	1.79	1.58
465	345	448		1.47	2.05	1.68
327	319	378		3.80	0.74	0.84
293	310	353		7.90	0.89	1.05
378	387	380		6.10	1.16	1.26
			C			
655	500	740		1.47	2.36	1.95
448	483	605		1.90	2.74	2.63
724	483	690		2.32	3.26	2.68
465	448	535		5.37	1.16	1.37
430	448	483		11.60	1.58	1.69
603	550	605		8.95	1.89	2.00

Lipids of Cod Muscle and the Effect of Frozen Storage[1]

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ABSTRACT

The lipid composition of fresh and frozen cod (*Gadus morhua*) muscle was studied using silicic acid chromatography. Dark cod muscle contained about three times as much total lipid as white muscle but the composition was quite similar. The most significant difference was that the dark tissue lipid contained more esterified cholesterol and less phosphatidyl choline. Frozen storage for up to 9 months at –12 C showed that the free fatty acid content increased from 5 to 326 mg/100 g tissue due to the hydrolysis of phosphatidyl ethanolamine and phosphatidyl choline. Other phospholipids were not hydrolyzed. Phosphatidyl ethanolamine hydrolysis ceased after storage for 4 months, whereas hydrolysis of phosphatidyl choline continued thereafter at a slower rate. After 9 months, the phospholipid content of the total lipid had dropped from 84 to 32% and only 13% of the original phosphatidyl ethanolamine and phosphatidyl choline remained unhydrolyzed.

INTRODUCTION

The preservation of fishery products by freezing has increased markedly in recent years and indications are that it will become even more important in the future. However, texture deterioration resulting from protein denaturation in certain stored-frozen products, particularly cod fillets, is a major problem which is not yet fully understood.

Dyer and Fraser (1959) postulated a relationship between protein denaturation and lipid hydrolysis in frozen cod fillets. Their data showed that lipid hydrolysis preceded protein denaturation and there was some indication that a critical level of free fatty acid (FFA) may be required to trigger protein denaturation. Workers in the United States (King et al., 1962; Anderson et al., 1963; Anderson and Steinberg, 1964; Anderson et al., 1965) have shown that cod actomyosin can be rendered insoluble in dilute salt solution by the addition of FFA beyond a threshold level which appears to be dependent upon the lipid content and the *p*H of the particular fish tissue. Hansen and Olley (1965) have concluded that the neutral lipids of the fish tissue are important in this connection and may be responsible for the differences in critical FFA level required to effect protein denaturation. A study by Olley and Duncan (1965) indicated more rapid protein denaturation in species wherein greater quantities of the more highly unsaturated FFA were liberated during frozen storage.

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It was known that phospholipids are the major lipid constituents of cod muscle and that their hydrolysis is responsible for the large increases in FFA in the frozen tissue (see Lovern et al., 1959; Olley and Lovern, 1960; Bligh, 1961; Olley et al., 1962). This paper records a quantitative study on the lipid composition of cod muscle and the changes occurring in the lipid constituents during frozen storage.

EXPERIMENTAL

In these experiments total lipid extracts prepared from samples of Atlantic cod fillets that had been stored at –12 C for 0, 1, 2, 4, 7, and 9 months were analyzed using chromatographic and chemical methods in order to quantitatively assess the changes that occur during frozen storage.

Cod Fillets

Freshly frozen cod fillets which had been prepared commercially were stored at the Laboratory in a constant-temperature cabinet maintained at –12 C, a temperature at which cod muscle lipid and protein soon deteriorate. The fish had been taken by commercial trawlers and were processed on the following dates: 1- and 2-month samples, November 15, 1961; 4-month sample, August 1, 1961; 7-month sample, April 15, 1961; 9-month sample, February 15, 1961. All of these fish had been caught a few days prior to these dates and held in ice aboard the trawlers until filleted and frozen. The initial sample (0 day) was taken from a live cod (caught September 22, 1961) that had been killed by a blow on the head and the tissue was in rigor at the time of lipid extraction.

To compare the composition of the total lipid from dark and white cod muscle tissue with that from whole cod fillets, a sample of dark muscle (115 g) was carefully dissected from a number of commercial fillets (processed May 31, 1961). Lipid extracts from composite samples of dark and white tissue were subsequently prepared for analysis.

Solvents

The following solvents were used: chloroform, reagent grade; methanol, absolute, reagent grade; ether, anhydrous, reagent grade; hexane, reagent grade, and dried over sodium.

Lipid Extraction

Total lipid was extracted from 400-g samples of tissue using the procedure of Bligh and Dyer (1959) and the tissue residue was washed with additional chloroform. The lipid content of each extract was determined by evaporating a 25-ml aliquot in a tared flask under a stream of nitrogen at less than 25 C. After drying in a vacuum desiccator over phosphorus pentoxide and paraffin wax, the dry weight was determined immediately using a Mettler semi-micro analytical balance. For column chromatography, portions of the extracts containing approximately 1.0 g total lipid were evaporated to dryness under reduced pressure (nitrogen) at less than 25 C. Whenever lipid samples had to be stored they were taken up in chloroform and stored under nitrogen at 0 C.

Silicic Acid Chromatography

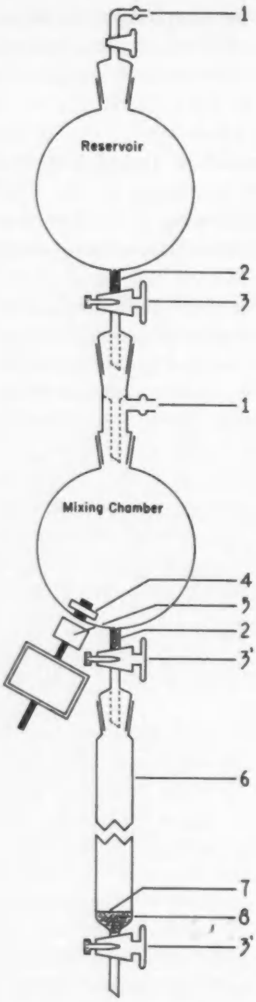
From information derived from the literature (see Lakshamanan and Lieberman, 1954; Hirsch and Ahrens, 1958; Hanahan et al., 1957; Wren, 1960) and after some experimentation, the following procedure was used to fractionate the lipids of cod muscle by gradient elution chromatography on silicic acid.

For each column, 100 g Mallinckrodt silicic acid (100-mesh, "suitable for chromatographic analysis by the method of Ramsay and Patterson") which had been heated overnight at 115 C, was shaken in a glass-stoppered cylinder with 1000 ml ether. After settling for 5 min, the supernatant liquid containing the fine particles was discarded; the remaining slurry was transferred to a sintered-glass funnel and washed four times by filtration with each of the following solvents (250 ml used for each wash): 4:1 chloroform–methanol; chloroform; and hexane. The residue was transferred with 250 ml hexane to a round-bottom flask and the flask evacuated for several minutes to remove air from the slurry. Nitrogen was then admitted into the flask. After the slurry had come to room temperature, it was poured into the chromatographic column.

Approximately 1.0 g total lipid was applied to the column in 10 ml hexane and was washed in with a total of 50 ml hexane. Another 50 ml hexane were added to the top of the column and automatic collection of 10-ml fractions in tared test tubes was commenced.

Using the apparatus shown in Fig. 1, elution with an increasing concentration gradient of ether in hexane followed by the same concentration gradient of methanol in chloroform was carried out as follows. With 600 ml hexane in the *mixing chamber* and about 500 ml ether in the *reservoir*, the flow rate of the column was maintained at 3–4 ml/min (R_2) with slight nitrogen pressure. The rate of entry of ether into the mixing chamber (R_1) was adjusted and maintained at one-third R_2. Thus the concentration gradient of ether in hexane would be represented by a concave curve upwards (see Lakshamanan and Lieberman, 1954). When the mixing chamber became empty at about fraction 85, the remaining ether was discarded and 700 ml chloroform was put into the mixing chamber and 1000 ml methanol in the reservoir. After 100 ml chloroform had entered the column (10 fractions later), methanol was allowed to drip into the mixing chamber and R_1 maintained at one-third R_2. When the mixing chamber again became empty, the remaining methanol was allowed to flow into it and elution was completed with pure methanol.

Each 10-ml fraction was evaporated to dryness under nitrogen (see above) and weighed to the nearest hundredth of a milligram. Fraction weights were plotted against fraction number and fractions representing distinct lipid components were pooled in chloroform for subsequent analysis. Total lipid recovery averaged 98%.



1. To nitrogen supply (1–4 cm Hg).
2. Cotton filter.
3. Teflon stopcock with metering valve (flow, R_1).

3'. Same as (3) (flow, R_2), these stopcocks normally left wide open.

4. Stirring magnet with polyethylene collar.
5. Magnetic stirrer.
6. Chromatographic column (3 cm ID and about 50 cm long) jacketed and maintained at 22 C.
7. Filter paper disc.
8. Glass wool.

FIG. 1. Apparatus for gradient elution chromatography; reservoir and mixing chamber 1000 ml capacity.

ANALYSES

Chemical and qualitative chromatographic analyses were carried out as follows: lipid phosphorus (Harris and Popat, 1954); lipid nitrogen (Lang, 1958); cholesterol (Foldes and Wilson, 1950); total choline (Entenman et al., 1944); lecithin choline (Hack, 1947). Sterols were detected using the Lieberman-Burchard reagent; 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid were added to the lipid sample in 2 ml chloroform. For free fatty acids, the lipid was dissolved in 1:1 chloroform–methanol and titrated with sodium methylate in methanol (1 ml equivalent to 10 mg fatty acid average mol wt of 300) using an automatic titrator. Serine and ethanolamine were determined according to Dittmer et al. (1958) with the exception that nitrogen was determined using the microdiffusion method of Conway (see Conway, 1962) followed by nesslerization.

Qualitative assessment of phospholipid choline, ethanolamine, and serine was done according to the hydrolytic and chromatographic procedures outlined by Marinetti et al. (1957). A spray of 0.2% ninhydrin in 95% ethanol containing a few drops of collidine followed by heating at 80 C for 20–30 min was used to detect compounds containing free amino groups. In the choline test, 1% aqueous phosphomolybdic acid was substituted for the chloroform–methanol reagent. Glycerol and inositol were detected in the hydrolysates using the paper chromatographic system of Hanahan and Olley (1958) followed by treatment with Trevalyan's reagent as recommended by Anet and Reynolds (1954). Phospholipids were further identified using the hydrolytic and chromatographic procedures of Dawson (1960).

RESULTS AND DISCUSSION

Lipid Composition of Cod Muscle

The chromatographs showing the fractionation of total lipid from whole cod fillets (0 day storage), and dark and white muscle are given in Fig. 2. The gradient elution system was an improvement over that used previously (Bligh, 1961) and gave good separation of the various lipid constituents and reproducibility was excellent. The initial increasing gradient of ether in hexane separated the neutral lipids into four distinct "components" and subsequent elution with an increasing gradient of methanol in chloroform followed by 100% methanol gave four phospholipid "components." The fractions making up the eight components were pooled as indicated in Fig. 2. On the basis of elution characteristics, and chemical and chromatographic analyses, they were identified as follows:

Component I consistently gave a positive Lieberman-Burchard test and esterified cholesterol represented the bulk of the total weight. Although this component would contain hydrocarbons and waxes, chemical analyses indicated the presence of only very small amounts of these compounds (see Table II).

Component II was identified as triglyceride. It gave a negative Lieberman-Burchard test, yielded glycerol on hydrolysis, and did not contain FFA (in frozen samples containing larger amounts of FFA, components II and III overlapped).

Component III was devoid of sterol and chemical analysis showed it to be FFA.

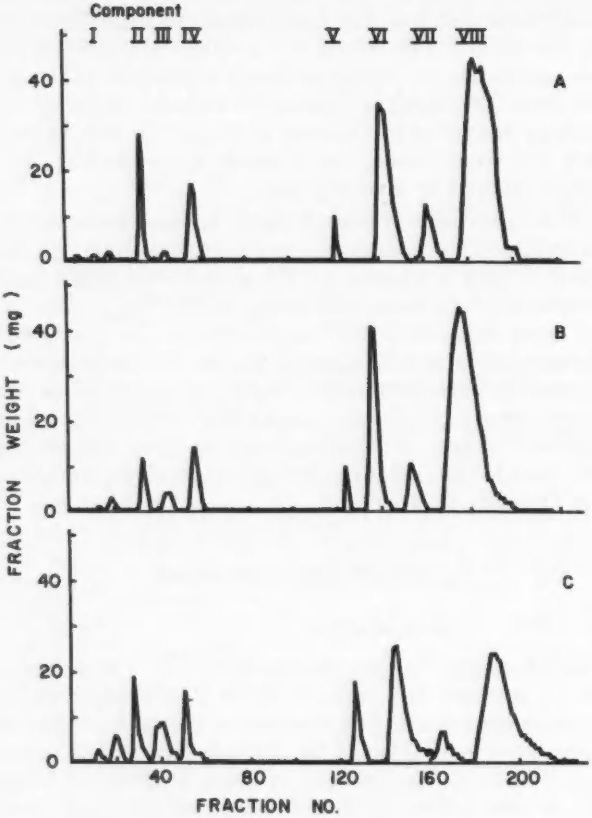


FIG. 2. Separation of lipid components from (A) whole cod fillets (0 day storage), and (B) white and (C) dark muscle using silicic acid chromatography.

Component IV was considered to be practically pure cholesterol. It reacted strongly with Lieberman-Burchard reagent and gas chromatographic analysis on an SE-30 column (whole fillet sample) showed only one peak corresponding to cholesterol.

Component V was not identified but could contain polyglycerol phosphatide and phosphatidic acid.

Component VI was primarily phosphatidyl ethanolamine with about 5–10% phosphatidyl serine. This component was exceptionally prone to oxidation.

Component VII on acid hydrolysis gave ethanolamine, serine, and inositol. Hydrolysis according to Dawson (1960) showed that the ethanolamine was largely present as plasmalogen. Only a small amount of inositol was present and the proportion of ethanolamine to serine was about 3:1.

Component VIII contained phosphatidyl choline as the major phospholipid with about 10% sphingomyelin.

Quantitative values for the various lipid components shown in Fig. 2 are compared in Table I. Although the dark tissue contained about three times as much lipid as the rest of the muscle, the composition was surprisingly similar. Esterified cholesterol, triglyceride, and phosphatidyl ethanolamine were somewhat higher in the dark muscle lipid whereas phosphatidyl choline was lower.

The lipid content of the dark tissue was considerably higher than expected in view of values recorded by Olley and Lovern (1960) for subcutaneous layers of cod which would contain both dark and white tissue. However, their finding that the lipid from these layers contained less phosphorus and more cholesterol than that from more interior layers is in agreement with the values in Table I. The dark and white tissue lipids contained 77.3 and 91.4% phospholipid, respectively (whole fillet, 84.0%). The major difference was in phosphatidyl choline content. These values represent 1.7 and 0.65 g phospholipid per 100 g dark and white tissue, respectively, in agreement with earlier reports that dark tissue is richer in phospholipid (Olley et al., 1962; Lovern, 1962).

The triglyceride content of the dark muscle lipid made up a minor part of the total lipid and the difference from that of the white muscle was not as marked as anticipated.

TABLE I. Composition of lipid from whole cod fillets compared to that for dark and white cod muscle tissue (as per cent of total lipid except for "total lipid").

Lipid component	Whole cod fillet[a]	Dark muscle	White muscle
Total lipid (*g/100 g tissue*)	0.75	2.20	0.71
I Cholesterol esters + trace of hydrocarbon	0.9	4.6	0.7
II Triglyceride	5.9	5.1	2.5
III FFA	0.7	7.0	1.8
IV Cholesterol	5.9	5.7	4.1
V Unidentified phospholipid	1.7	5.9	1.7
VI Phosphatidyl ethanolamine + about 7% phosphatidyl serine	17.9	20.7	16.6
VII Phosphatidal ethanolamine + phosphatidyl serine (about 3:1) + small amount inositol phosphatide	6.8	5.5	6.1
VIII Phosphatidyl choline + about 10% sphingomyelin	57.6	45.2	67.0

[a] See Table II for more detailed data.

The lipid of dark muscle contained more FFA than that from white muscle but these values may not be too significant since some hydrolysis could have occurred in the commercial samples prior to extraction. The FFA value 0.7% of total lipid for whole cod fillet (extracted in rigor) indicates that the FFA content in cod tissue lipid immediately after death may be lower than previously considered. Olley and Lovern (1960) reported values as low as 1.4% FFA in fillets rapidly frozen in solid CO_2.

LIPID HYDROLYSIS IN FROZEN COD MUSCLE

The chromatographic separation of the lipid components from samples of cod muscle stored at −12 C for 1, 2, 4, 7, and 9 months is shown in Fig. 3. As in previous experiments (Bligh, 1961), these chromatographs revealed that the major precursors of the FFA (component III) which develop in frozen cod were phosphatidyl ethanolamine (VI) and phosphatidyl choline (VIII). Also, it appeared that hydrolysis ceased after storage for 4 months at this temperature. The chemical analyses recorded in Table II were carried out to verify these points.

The cholesterol content of component I was similar to that found by Lovern et al. (1959) in fresh cod. However, conversion of the values to esterified cholesterol indicated that hydrocarbons and waxes could only be present in very small amounts.

The fresh cod sample was the only instance where component II was separated as a distinct peak. In this sample, the triglyceride content was 6% of the total lipid.

Results for component III show that the FFA content increased rapidly from 5 mg/100 g tissue to a plateau after 4 months storage around the 300 mg/100 g level reported by Olley et al. (1962).

Components IV and V remained unchanged throughout the storage period. The total cholesterol content for the six samples averaged 50 mg/100 g tissue of which about 80% was free (see Lovern, 1962).

Table II shows that only the phosphatidyl ethanolamine of component VI was hydrolyzed during frozen storage. During the first 4 months of storage, approximately 82 mg/100 g tissue of phosphatidyl ethanolamine were hydrolyzed. Hydrolysis appeared to cease thereafter even though about one-third of the phosphatidyl ethanolamine was still available. The loss of ethanolamine nitrogen was 1.5 mg which would be equivalent to the release of 64 mg FFA.

Neither phosphatidyl serine nor phosphatidal ethanolamine in component VII were hydrolyzed in the stored-frozen cod samples.

The results for component VIII show that phosphatidyl choline dropped rapidly for the first 4 months of storage but hydrolysis continued at a decreased rate for the remainder of the storage period. In 4 months, the loss of phosphatidyl choline would be equivalent to the liberation of about 253 mg FFA.

These analyses confirm the earlier report of Bligh (1961) that phosphatidyl ethanolamine and phosphatidyl choline are the only phospholipids hydrolyzed in cod muscle during frozen storage. After storage for 4 months at −12 C, 317

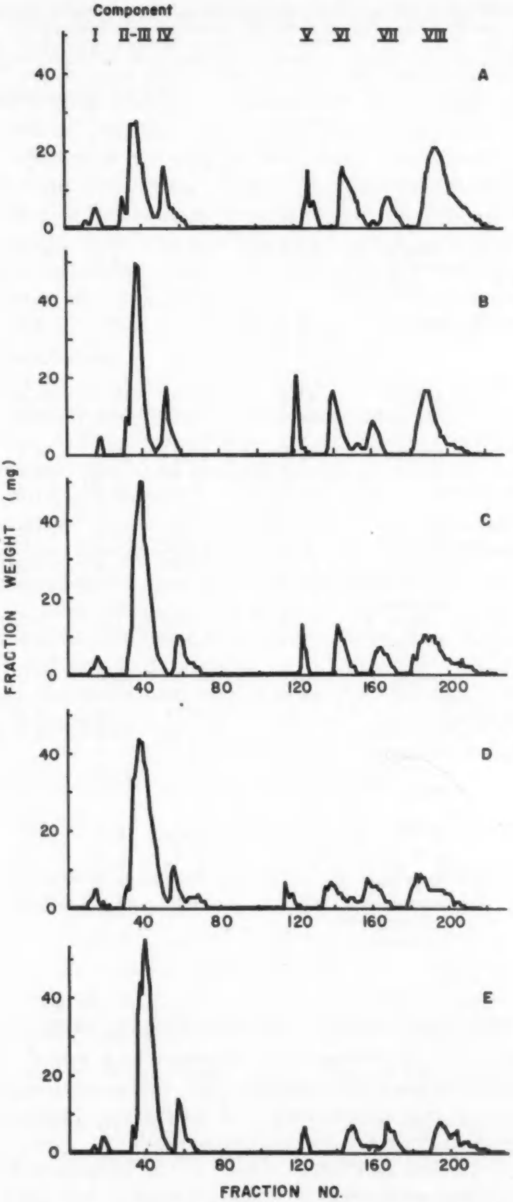


FIG. 3. Chromatographic separation of lipid components from cod muscle stored at −12 C for (A) 1, (B) 2, (C) 4, (D) 7, and (E) 9 months (see Fig. 2(A) for sample stored 0 day).

TABLE II. Analytical data on lipid components from frozen cod muscle stored at -12 C. (Values as mg/100 g tissue; those in parentheses are uncertain.)

		Storage time (*months*)					
		0	1	2	4	7	9
Total lipid		750	728	687	716	610	594
Component I	— weight	6.7	19.0	9.0	16.9	16.2	14.8
	— cholesterol (esterified)	3.0	11.2	5.5	10.3	10.1	8.5
Components II and III	— weight	43.8 (II)	176	250	329	327	340
	— weight	4.7 (III)					
	— FFA	5.1 (III)	180	240	326	316	310
Component IV	— weight	43.5	(67.6)	(64.2)	51.4	53.6	47.8
	— cholesterol	43.4	37.5	40.6	44.0	46.6	41.0
Component V	— weight	13.2	35.7	34.7	37.0	27.3	20.6
	— N	0.07	0.18	0.09	0.18	0.15	0.14
	— P	0.36	0.51	0.46	0.46	-	0.39
Component VI	— weight	134	124	72.1	54.4	49.9	53.2
	— N	2.35	2.02	(1.71)	0.99	(1.19)	0.88
	— P	(4.17)	3.99	2.97	2.05	1.68	(1.21)
	— N/P (*moles*)	(1.24)	1.12	(1.27)	1.08	(1.57)	(1.60)
	— ethanolamine-N	2.21	1.89	1.25	0.70	0.74	0.72
	— serine-N	0.18	0.20	-	0.10	0.14	0.11
Component VII	— weight	50.5	46.7	46.6	43.0	47.5	38.9
	— N	0.78	0.89	(0.63)	0.83	0.95	0.70
	— P	1.53	1.71	1.58	1.87	1.86	1.39
	— N/P (*moles*)	1.14	1.16	(0.87)	0.98	1.12	1.11
	— ethanolamine-N	0.49	0.65	(0.70)	0.56	0.49	0.49
	— serine-N	0.16	0.09	-	0.15	0.17	0.19
Component VIII	— weight	432	273	171	128	95.3	79.2
	— N	7.73	4.20	2.73	2.35	1.78	1.44
	— P	12.5	7.56	4.82	3.66	2.81	1.64
	— N/P (*moles*)	1.38	1.23	1.25	1.43	1.41	1.94
	— total choline	67.2	35.1	23.2	16.5	13.6	9.5
	— sphingomyelin choline	(6.0)	3.4	1.7	-	1.0	2.9

mg FFA were liberated through the hydrolysis of these compounds. This figure agrees well with the 326 mg FFA isolated which would include any FFA derived from triglycerides. In accordance with Olley and Lovern (1960), there was no evidence of the accumulation of hydrolytic intermediates such as diglycerides, phosphatidic acids, or lysophosphatides.

As stated by Lovern and Olley (1962), it has been recorded for a variety of conditions that the hydrolysis of phospholipids in cod muscle slows down markedly after about 75% of the original phospholipids have been hydrolyzed. In these experiments at -12 C, the loss of phospholipid through hydrolysis after storage for 1, 2, 4, 7, and 9 months was 24, 49, 60, 65, and 70%, respectively. (During this period, the phospholipid content of the total lipid dropped from 84 to 32%.) These figures show that hydrolysis began to slow down when only 60% of the original phospholipids had been hydrolyzed (after 4 months). In fact, hydrolysis of phosphatidyl ethanolamine appeared to cease at this level. However, phosphatidyl choline hydrolysis continued up to at least 9 months. By this time, 70% of the original phospholipids had been hydrolyzed. Since only phosphatidyl ethanolamine and phosphatidyl choline are hydrolyzed, and they account for 83% of the original phospholipids, this means that of the phospholipids remaining only 13% can be hydrolyzed. At 4 months, the corresponding figure would be 23%, or when 72% of the hydrolyzable phospholipid had been used up. Thus, it would be reasonable to expect a slowing down of the rate of hydrolysis.

The cessation of hydrolysis of phosphatidyl ethanolamine while phosphatidyl choline hydrolysis continued suggests that separate enzymes are involved and that one becomes inactivated before the other. Yurkowski and Brockerhoff (1965) found that oleic acid had a strong inhibitory effect on the lysolecithinase activity of cod muscle. This could support the concept of two enzyme systems, one of which might be inhibited more than the other. On the other hand, and based on findings by Olley and Duncan (1965), the composition of the remaining phosphatidyl ethanolamine may be unsuitable for further enzyme hydrolysis. As noted above, this component was very prone to oxidation and might therefore have become oxidized and rendered inactive. However, Olley and Duncan (1965) did not find evidence of preferential oxidation of the more susceptible fatty acids in frozen fish tissues. An alternate concept, suggested by Olley (personal communication), is that one first-order hydrolytic reaction proceeds for the first 4 months becoming asymptotic, and that a different reaction proceeds thereafter at a much slower rate.

ACKNOWLEDGMENTS

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Resistance to Zinc Sulphate of Zebrafish (*Brachydanio rerio*) Embryos After Removal or Rupture of the Outer Egg Membrane[1]

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ABSTRACT

Fifteen- and 25-hr-old zebrafish (*Brachydanio rerio* Hamilton-Buchanan) embryos with the outer egg membrane ruptured survived longer in a solution of zinc sulphate (20 ppm Zn) than embryos of the same ages with the membrane intact. Forty-two-hour embryos with the membrane completely removed survived in four concentrations of zinc sulphate (2.5, 5, 10, and 20 ppm Zn) at least as long as 42-hr embryos with the membrane entire. The high resistance of zebrafish embryos to zinc was therefore not owing to protection by the membrane. On the contrary the presence of the membrane lowered resistance.

This result was probably owing to the formation of opaque material enclosed by the outer egg membrane of zinc-poisoned, unruptured eggs. The action of this material in reducing survival is unknown.

INTRODUCTION

It has been observed (Skidmore, 1965) that survival time of the zebrafish (*Brachydanio rerio* Hamilton-Buchanan) in zinc sulphate solution (containing 5, 10, 20, or 40 ppm zinc as Zn) decreases steadily with age from time of laying until time of hatching, and then remains substantially the same for several days. A partial explanation of this could be that the resistance of the unhatched embryo is initially high because the outer egg membrane (as defined by Domurat, 1964) protects it in some way, possibly by excluding some of the zinc. Decline in resistance with age may result from progressive increase in permeability by the membrane, which was observed to become increasingly brittle as embryological development proceeded. Most embryos hatch on the third day at 25 C.

If the membrane does give some protection from a solution of zinc sulphate, it follows that its removal or rupture would decrease the survival time of the embryo. The present study was intended to test this hypothesis, although the result was the opposite of that expected.

MATERIALS AND METHODS

Zebrafish eggs from a single pair of fish were reared in Canberra tap water (total hardness 10 ppm as $CaCO_3$), at 25 C, *p*H 7, and dissolved oxygen concentration at least 6 ppm, as described previously (Skidmore, 1965). Mortality

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during rearing was less than 10%. Rates of development were similar to those described by Hisaoka and Battle (1958).

Thirty hours after laying, soon after the heart began to beat, the outer membranes of 50 eggs were removed, using fine scissors and forceps. The naked embryos were then maintained in tap water for 12 hr, to detect deaths resulting from mechanical or other damage prior to testing. None in fact occurred. Two 31-hr-old embryos are illustrated; one with its membrane removed (Fig. 3), the other with its membrane intact (Fig. 4).

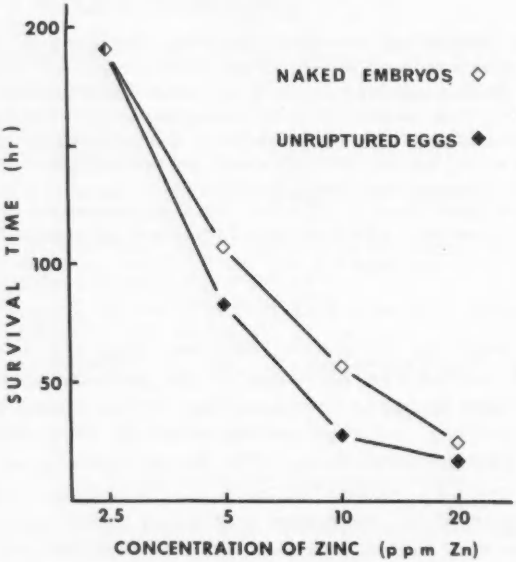


FIG. 1. Survival of naked zebrafish embryos and embryos in unruptured eggs, all 42 hr old, when exposed to four concentrations of zinc sulphate in Canberra tap water. Each point represents the median value for 10 embryos (experiment 1).

Forty-two hours after laying, samples of ten naked embryos from the above treatment were transferred to each of four test solutions of zinc sulphate in tap water, containing 2.5, 5, 10, and 20 ppm zinc. Ten of these embryos were maintained in tap water as controls. Concurrently, samples of 10 unruptured eggs were transferred to similar zinc sulphate solutions, and again 10 control animals were set aside. Bioassay procedures used here and throughout the tests were as described previously (Skidmore, 1965). Individual times to immobilization were recorded with an error of less than 20%. No control animals died.

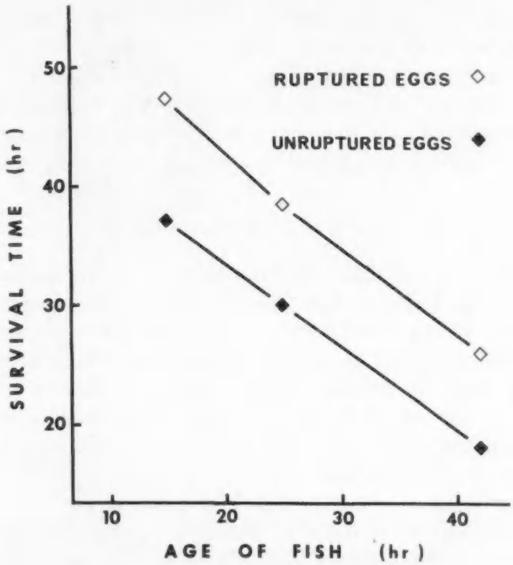


FIG. 2. Survival of embryos in ruptured zebrafish eggs (plus naked embryos) and those in unruptured eggs, of three different ages, when exposed to zinc sulphate (20 ppm Zn) in Canberra tap water. For 15- and 25-hr-old embryos, each point represents the median value for 30 embryos (experiment 2). For 42-hr-old embryos, each point represents the median value for 10 embryos (experiment 1).

In a second experiment, a further batch of eggs was similarly bred and reared. Mortality was again less than 10%. Ten hours after laying, and shortly after closure of the blastopore, attempts to remove the outer egg membrane failed. Because of the toughness of the membrane, the embryo was damaged every time. As the membrane could not be completely removed, alternatively, it was ruptured by grasping a small fold of membrane with two fine forceps and pulling the forceps apart (Fig. 5). Care was taken to exert no pressure on the embryo. With practice, a tear could be made in the membrane, equivalent to approximately 10% of its total surface area. Seventy-seven eggs were successfully treated in this way between 10 and 13 hr after laying. Ruptured and unruptured eggs, 14 hr old, are illustrated in Fig. 6 and 7. The 77 ruptured eggs, plus approximately 100 unruptured eggs, were maintained alive in tap water for several hours; none died. Fifteen and 25 hr after laying (after development of optic vesicle and 20 somites, respectively), samples of 30 ruptured and 30 unruptured eggs were transferred to a 20 ppm zinc solution in tap water. The individual survival times of all 120 embryos were noted, with an error of less than 10% in each case. No control animals died.

In both experiments, the median survival times of samples of embryos exposed to similar conditions were estimated by probit analysis (Bliss, 1937). Median survival times of naked embryos and embryos in ruptured eggs were compared with those of embryos in unruptured eggs, all data from each experiment being considered concurrently. The statistical significance of differences between related medians was tested by the analysis of variance.

RESULTS AND DISCUSSION

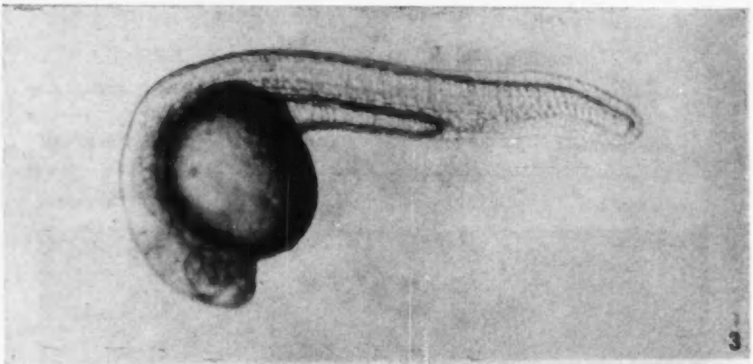
The median survival times of samples of 42-hr-old embryos (experiment 1) are shown in Fig. 1. In 2.5 ppm zinc, survival times of naked embryos and those in unruptured eggs were similar. This was the expected result because survival times extended far beyond hatching, after which event all fish (now alevins) would naturally be naked. However, in all higher concentrations of zinc, naked embryos tended to survive longer than those with the outer egg membrane intact, but the difference was not statistically significant ($P = 0.1$).

Figure 1 demonstrates that the resistance to zinc of naked zebrafish embryos was at least as great as the resistance of embryos in unruptured eggs. The hypothesis that removal of the outer egg membrane would decrease the survival time of the embryo must therefore be rejected. The alternative hypothesis, that removal or rupture of the membrane would increase the survival time of the embryo, will next be considered. Because the differences between treated and untreated eggs in experiment 1 were not statistically significant, experiment 2 was designed to compare the median survival times of much larger samples of two age-groups of younger embryos, which would be observed more frequently.

The results of experiment 2 are given in Fig. 2. (For purposes of comparison, the values for 42-hr embryos are repeated from Fig. 1.) Embryos of ruptured eggs, aged 15 and 25 hr, survived about 10 hr longer than embryos of unruptured eggs, so that the two curves are roughly parallel. This suggests that the effect of rupturing (in prolonging survival time) may be independent of age. The difference between the median survival times of embryos of ruptured eggs and those of intact eggs in experiment 2 was highly significant ($P < 0.001$, Table I). The following explanation for this result is offered.

The first sign that the embryo of an intact egg was dying in a solution of zinc sulphate was the formation of opaque patches within the fluid which bathes the embryo (Fig. 8). The patches gradually increased in number and size until they coalesced to surround the living embryo (Fig. 9). Later, the tissues of the embryo itself turned opaque, beginning with those in the tail. Finally the animal became immobilized, that is to say it ceased to move and its heart stopped beating. An unaffected embryo is shown for purposes of comparison in Fig. 10.

A naked embryo or an embryo in a ruptured egg did not become enclosed in a mass of opaque material when exposed to a toxic solution of zinc because the fluid surrounding the embryo had become lost to the environment. The



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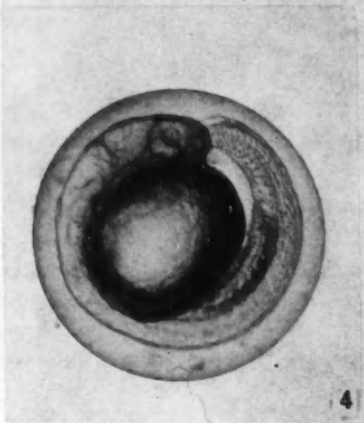

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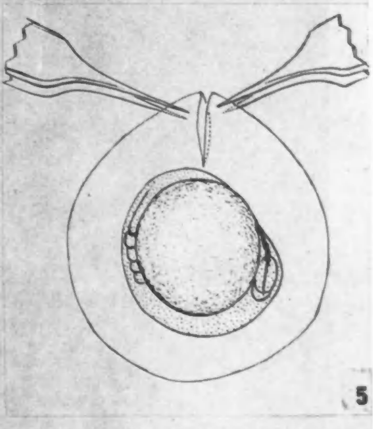

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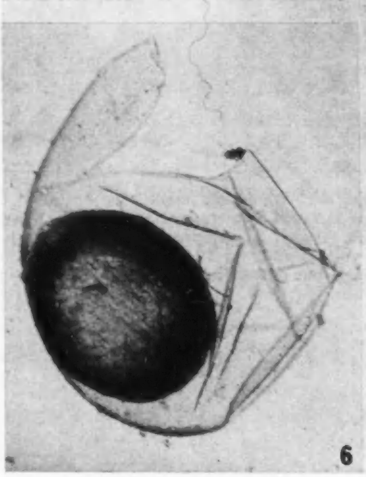

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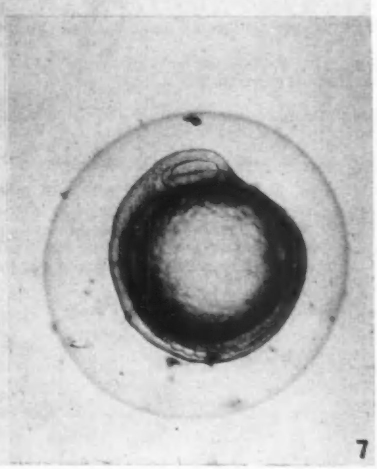

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FIG. 3–7. **3.** A 31-hr-old zebrafish embryo with outer egg membrane removed. **4.** A 31-hr embryo with membrane entire. **5.** Drawing of 13-hr embryo showing method of rupturing the membrane. **6.** A 14-hr embryo with membrane ruptured. **7.** A 14-hr embryo with membrane intact (× 40).

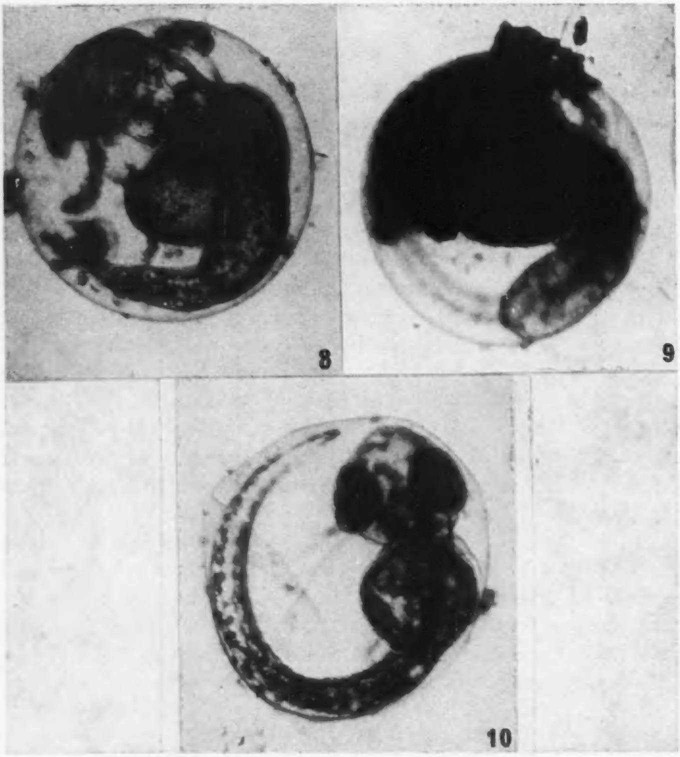

Fig. 8–10. **8**. A 64-hr zebrafish embryo with outer egg membrane entire exposed to 40 ppm zinc for 34 hr. Opaque material forming inside membrane. **9**. A 53-hr embryo with membrane entire exposed to 40 ppm zinc for 50 hr. Opaque material surrounding head and yolk of embryo. **10**. A 64-hr embryo reared in tap water. Membrane unruptured but dented by handling (× 40).

TABLE I. Analysis of variance of data in experiment 2.

Source of variance	Degrees of freedom	Sums of squares	Mean squares
Embryos[a]	1	4,795	4,795
Groups[b]	1	1,235	1,235
Embryos × groups	1	457	457
Replicate error	116	27,877	240
Total	119	34,364	—

[a] Embryos in ruptured or unruptured eggs.
[b] Age-groups, 15 or 25 hr.

embryo did however turn opaque in a similar manner to an embryo in an intact egg. Comparable changes developed appreciably later in naked embryos and in ruptured eggs.

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Ocean Migration and Distribution of Fin-Marked Coho Salmon[1]

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ABSTRACT

Migration, distribution, and movement of five groups of fin-marked coho salmon of the 1950 brood planted into Puget Sound, Washington, in 1952, were followed during 1952 and 1953 by a study of 847 recoveries of marked salmon taken in sport and commercial fisheries in the Pacific Ocean, Puget Sound, and closely adjoining regions.

Differences in migration, distribution, and movements of the five groups of salmon were correlated with the time, place, and method of planting.

The most pronounced difference in behavior was shown by salmon receiving an extra 2 months of rearing in the hatchery as compared with other groups. This group was marked by removal of the dorsal and right ventral fins. A lag in average time of migration to points in the ocean and a tendency to migrate lesser distances while in the ocean characterized the behavior of these fish. This lag in time of migration as indicated by patterns of recovery of marked salmon from sport and commercial fisheries was confirmed by an actual lag in time of return to the point of release.

In migrating from off the coast of Washington to Puget Sound, the salmon moved at an estimated rate of 5–7 nautical miles per day.

INTRODUCTION

In the spring of 1952, coho salmon (*Oncorhynchus kisutch*) marked by fin excision were released into streams entering southern Puget Sound (Minter Creek) and middle Puget Sound (Lake Washington drainage) (Fig. 1). This paper describes the migration and distribution in salt water of these salmon by analyzing the time and place of recovery of marked fish obtained from sport and commercial coho salmon fisheries in 1952 and 1953. Such knowledge of migratory paths is necessary to the management of salmon based upon local self-perpetuating populations of spawning fish (Rich, 1939; Thompson, 1959).

DESCRIPTION OF MARKED SALMON, METHODS OF RECOVERY, AND ANALYSIS OF MARKS

Experimental groups of salmon were released into the Lake Washington drainage in a cooperative study between the University of Washington College of Fisheries and the Washington State Department of Fisheries (Donaldson and Allen, 1958). This study was designed to measure the mortality of smolts

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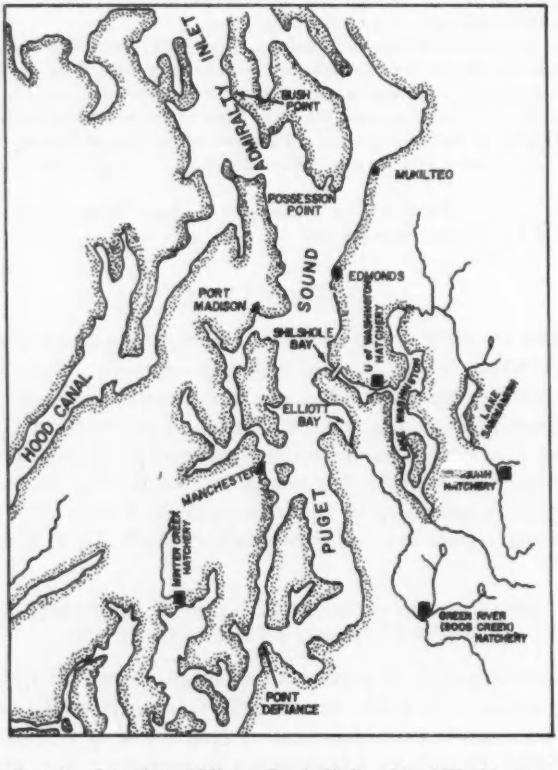
migrating through two lakes in the Lake Washington drainage. Salmon released into Minter Creek were part of experiments to measure survival of artificially propagated salmon associated with methods of planting salmon and differences in age at release (Salo and Bayliff, 1958). The Minter Creek experiment was conducted by the Washington Department of Fisheries. Details of these experiments are listed in Allen (1965, table 1).

Coho salmon planted at the University of Washington and Issaquah hatcheries were from Soos Creek (Green River) hatchery stock. These fish were reared at Soos Creek. Salmon planted into Minter Creek were a mixture of stock native to Minter Creek and salmon from Green River (Soos Creek) (Fig. 1). These salmon were reared at Minter Creek. Most fingerling salmon planted in the five groups were from 8 to 11 cm fork length. Dorsal-right ventral (D-RV) fin-marked salmon planted in Minter Creek were largest in average length of the five groups (Allen, 1959b, figure 2).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PORT MADISON
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LAKE WASHINGTON
LAKE SAMMAMISH
ELLIOTT BAY
HOOD CANAL
MANCHESTER
ISSAQUAH HATCHERY
MINTER CREEK HATCHERY
PUGET
GREEN RIVER (SOOS CREEK) HATCHERY
POINT DEFIANCE

FIG. 1. Location of Minter Creek, University of Washington, and Issaquah hatcheries, and of other points in Puget Sound region mentioned in text.

Each group of salmon was unique in some aspect of time, place, location, or method of release. For brevity these groups will be designated by the fin excision used to identify it. The italicized portions in Table I summarize the unique feature associated with each group.

Recoveries of marked salmon from the five releases were obtained from 17 different sources. The number of salmon recovered, the estimated catch in the fisheries sampled, and the number of fish in the catch examined for marked salmon are listed in Allen (1965, table 2). There were 847 recoveries available for analysis.

TABLE I. Experimental design associated with fin-marked coho salmon of 1950 brood year planted into Puget Sound, Washington, 1952.

		Experimental design		
Fin excised[a]	Point of release	Difference in location between experiments	Differences within experiments	Number salmon released
LV	University of Washington	*Middle Puget Sound* drainage	Control	34,405
RV	Issaquah	Middle Puget Sound drainage	Point of release *inland*[b]	36,833
Ad-An	Minter Creek	South Puget Sound drainage	*Scatter-planted* (released at multiple points)[c]	69,901
An-RV	Minter Creek	*South Puget Sound* drainage	Control	70,099
D-RV	Minter Creek	South Puget Sound drainage	*Two months older* at time of release[d]	96,620

[a] LV: left ventral; RV: right ventral; Ad-An: adipose anal; An-RV: anal-right ventral; D-RV: dorsal-right ventral.

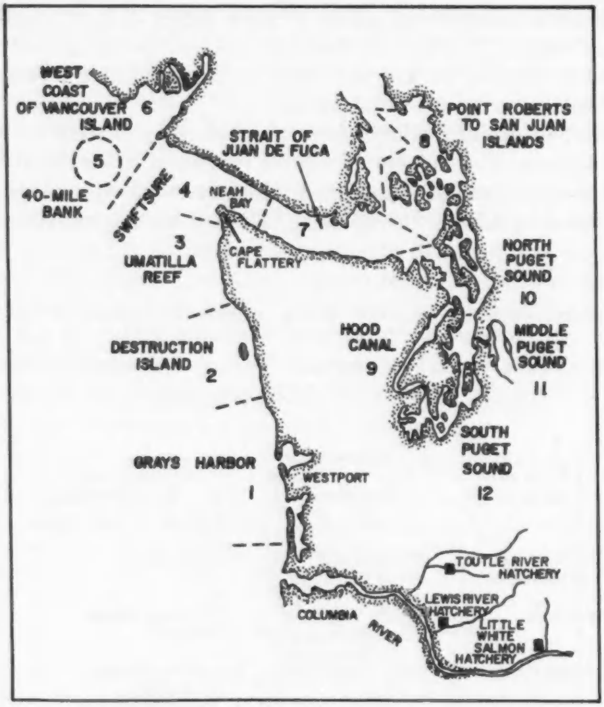
[b] Issaquah is located 35 miles inland whereas other points of release were all within 5 miles of Puget Sound. Smolts from Issaquah passed through three lakes before entering Puget Sound.

[c] Groups other than Ad-An were all released at a single point (mass-planted).

[d] Groups other than D-RV were all released in March. The D-RV group was planted in May.

Migration and distribution in waters adjacent to the State of Washington were studied by analyzing the data arranged with reference to 12 arbitrarily defined areas (Fig. 2). Areas 1 through 6 were used in analyzing marked salmon recovered in ocean troll fisheries. Areas 7 through 12 were used for recoveries made in sport and commercial fisheries within Puget Sound and closely adjoining regions.

Coho smolts entering Puget Sound will either migrate to the Pacific Ocean or remain within Puget Sound. The salmon remain in these areas during their second year of feeding in salt water. For salmon feeding in the Pacific Ocean


WEST COAST OF VANCOUVER ISLAND
6
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40-MILE BANK
SWIFTSURE
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NEAH BAY
STRAIT OF JUAN DE FUCA
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POINT ROBERTS TO SAN JUAN ISLANDS
8
3
UMATILLA REEF
CAPE FLATTERY
NORTH PUGET SOUND
10
HOOD CANAL
9
MIDDLE PUGET SOUND
11
DESTRUCTION ISLAND
2
SOUTH PUGET SOUND
12
GRAYS HARBOR
1
WESTPORT
TOUTLE RIVER HATCHERY
LEWIS RIVER HATCHERY
LITTLE WHITE SALMON HATCHERY
COLUMBIA RIVER

FIG. 2. Areas used in analyzing recoveries of marked 1950 brood coho salmon released into Puget Sound, and location of salmon hatcheries on the Columbia River mentioned in text.

rapid growth takes place during July, August, and September (Allen, 1959b, figure 4). Migration to points of release in fresh water starts during the fall of the second year in salt water.

In 1952, data on marked salmon were obtained from two sources only. Some marked salmon had grown large enough to enter the sport fishery by the fall of the year and were reported voluntarily by sportsmen using postcards. Most marked salmon recovered in 1952 were taken by biologists fishing a commercial reef net at Bush Point in northern Puget Sound (Fig. 1).

In 1953, marked salmon were reported from many areas and sources. Marked salmon remaining within Puget Sound (inside fish) were obtained by biologists sampling the sport fishery and from voluntary reporting on postcards by sportsmen. Salmon migrating to feeding grounds in the Pacific Ocean were taken in large numbers by commercial troll fisheries. Some marked fish were reported by postcards from sport fisheries in the Pacific Ocean and Juan de Fuca Strait. Commercial gillnet and purse-seine fisheries in Puget Sound took substantial numbers of marked salmon in the late summer and fall as the salmon were returning to their points of release.

MIGRATION AND DISTRIBUTION IN PUGET SOUND

Marked 1950-brood coho fingerlings were released in March and May 1952. The first marked salmon were recovered in mid July during experimental fishing with a commercial reef-net at Bush Point (Fig. 1). Among the 7376 coho salmon taken in the summer of 1952 with this gear, 10 marked salmon were from Lake Washington and 63 were from Minter Creek. The number of D-RV-marked salmon recovered per thousand fish caught showed a very marked increase as compared to other groups during the latter part of the sampling (Fig. 3). This was interpreted as a definite lag in average time of arrival at Bush Point for the D-RV group.

There was indication of a later average time of migration into Puget Sound for the right ventral fin (RV) group as compared to the left ventral fin (LV) group. A few RV fish but no LV fish were taken during the August sampling at Bush Point (Fig. 1). The first LV marks reported were taken in July while the first RV marks were taken in October (Table II). Of the three RV marks reported in October, two were reported from Lake Washington. In contrast, LV marks reported in July and September all came from Puget Sound.

The pattern of recovery of RV and D-RV fish in 1952 reflected the conditions of release — a 2-month delay in time of release for the D-RV group and farther inland site of release for the RV group. It will be shown later that these lags in average time of arrival in 1952 were discerned at other stages of the migration to the Pacific Ocean and return to Puget Sound in 1953.

Fishing at Bush Point in 1952 was designed to capture immature coho salmon in their second year of life. From July 24 to August 22 all salmon caught were marked by removing the dorsal (D) and adipose (Ad) fins. Jensen (MS, 1954) reported 2.27% of these marked salmon were recovered, all recoveries being from points south of the northern boundary of area 10 (Fig. 2). The results indicated that the coho salmon in the Bush Point area during the late July–August period were not moving to offshore waters.

Distribution of salmon within Puget Sound during fall and winter 1952 was indicated by marked fish reported by sporstmen (Table II). Two LV marks were reported, both of which were caught in north Puget Sound (area 10). Of four RV fish reported, two were caught south of Shilshole Bay (areas 11 and 12). Nine Minter Creek marks were reported, none of which was caught in north Puget Sound (area 10). Minter Creek fish were caught mainly in southern areas: south Puget Sound (anal fin, An) (Ad-An, 2; An-RV, 1; from Point Defiance, area 12); middle Puget Sound (Ad-An, 3; D-RV, 2; from Elliot and Shilshole bays, area 11); and from Hood Canal (An-RV, 1).

Distribution of the salmon during 1953 could be studied through marks obtained from sampling of the sport catch and by sportsmen's returns. Unfortunately, sampling of the catch in 1953 concentrated heavily on landings made at boathouses located near Point Defiance (area 12) and at Manchester, and Shilshole and Elliot bays (area 11). Catches made near Possession Point and in other parts of north Puget Sound (area 10) were very lightly sampled (Allen,

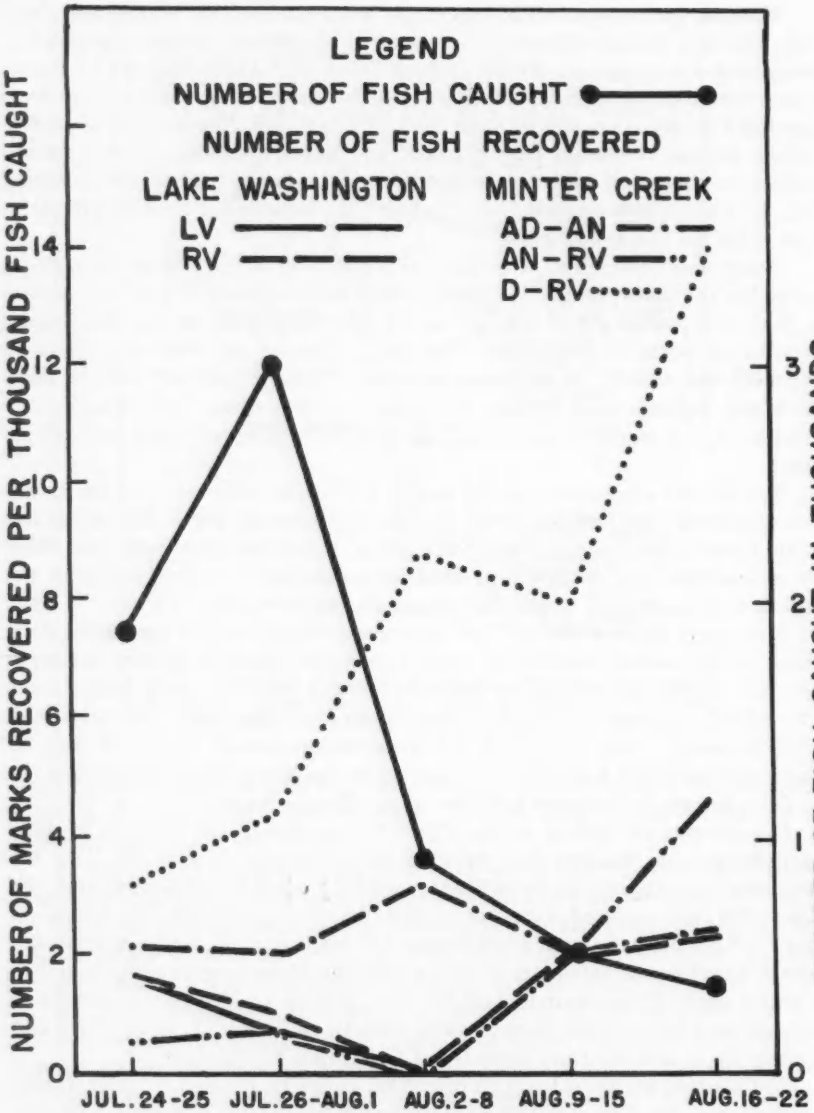

FIG. 3. Number of marked 1950 brood coho salmon per thousand salmon caught in reef-net fishing at Bust Point, Puget Sound, during July and August 1952 (from Jensen, 1954).

TABLE II. Number of fin-marked coho salmon reported by sportsmen by month to Washington State Department of Fisheries in 1952 and 1953.

Year and area of recovery	Fin mark	Month of recovery												Totals		
		J	F	M	A	M	J	J	A	S	O	N	D	By group	Lake Wash.	Minter Creek
1952, Puget Sound																
	LV							1		1				2		
	RV										3		1	4	6	
	Ad-An											1	3	4		
	An-RV										1	1		2		
	D-RV										1	2		3		9
	Total:							1		1	5	4	4	15	6	9
1953, Puget Sound																
Area 10	LV			1	1	1	1	2		3	1			10		
	RV					1		5	1	4	4	1		16	26	
	Ad-An			1				1	1					3		
	An-RV					1				3	2			6		
	D-RV				2	1	1	2	3	1				10		19
	Total:			2	3	4	2	10	5	11	7	1		45	26	19
Area 11	LV	2	1							1				4		
	RV	2	1	1	1.		3		1	1				10	14	
	Ad-An	1	5						1		2			9		
	An-RV	2	6	1										9		
	D-RV	6	4						1					11		29
	Total:	13	17	2	1		3		3	2	2			43	14	29
Area 12	LV							1						1		
	RV	1				1		1		1	1			5	6	
	Ad-An	1	2	1										4		
	An-RV	1	3											4		
	D-RV	1	1						1					3		11
	Total:	4	6	1		1		2	1	3	3			17	6	11
1953, Pacific Ocean and Juan de Fuca Strait																
	LV							6	4	5				15		
	RV	1					1	2	4	2				10	25	
	Ad-An													0		
	An-RV								1					1		
	D-RV								2	1				3		4
	Total:	1					1	8	11	8				29	25	4

MS, 1956, figure 40). Therefore sportsmen's returns arranged by month and by mark (Table II) and by area and by mark (Table III) were used to give some general idea on distribution within Puget Sound in 1953.

Whether error in sportsmen's returns would preclude such an analysis cannot be answered definitely. The ratio of biologists' to sportsmen's returns of 80:59 for double-fin marks and 11:46 for single-fin marks (Table III) might suggest that sportsmen reported single-fin marks at a higher rate than biologists. This would be a reasonable conclusion if marked fish in each group were distributed randomly throughout Puget Sound, *or* if the search for marks by sportsmen and by biologists were in the same ratio throughout the Sound. Neither of these conditions was satisfied, however. The uneven distribution of the biologists' search for marks was described above; the recoveries by biologists showed practically no single-fin marks in south Puget Sound (area 12) but did show a large number of double-fin marks (Table III). In area 11, where the biologists' recoveries were concentrated, the above ratios are 22:29 for double marks and 9:14 for single marks — not significantly different from each other. Thus sportsmen's returns were considered to be as good an indicator of distribution, despite the possibility of some error from wrongly reported marks, as could be obtained by trying to adjust biologists' returns.

TABLE III. Number of fin-marked coho salmon reported by sportsmen to Washington State Department of Fisheries and recorded by biologists sampling the catch of Puget Sound sport fishery in 1953.

Area of recovery	LV	RV	Total	Ad-An	An-RV	D-RV	Total
10	10 (0)[a]	16 (1)	26 (1)	3 (6)	6 (2)	10 (6)	19 (14)
11	4 (4)	10 (5)	14 (9)	9 (7)	9 (8)	11 (7)	29 (22)
12	1 (0)	5 (1)	6 (1)	4 (14)	4 (16)	3 (14)	11 (44)
Totals:	15 (4)	31 (7)	46 (11)	16 (27)	19 (26)	24 (27)	59 (80)

[a] Figures in parentheses are marked salmon recovered by biologists in sampling the catch.

Minter Creek fish definitely appeared to favor southern Puget Sound waters, whereas Lake Washington fish were more evenly distributed. This was indicated by both biologist and sportsmen returns (Table III). This could be more clearly shown by the ratios between sportsmen's recoveries from northern and southern areas in Puget Sound (Table IV). The RV and D-RV groups were more equally distributed in all parts of Puget Sound. The Ad-An and An-RV groups showed a more pronounced southern distribution while the LV group showed a more pronounced northern distribution.

Compared with other Minter Creek groups, D-RV fish seemed to favor the northern areas. D-RV fish not only were taken in greater numbers in area

TABLE IV. Ratio of marked salmon reported by sportsmen between northern and southern Puget Sound area in 1953.

Areas compared	Salmon planted into Lake Washington drainage			Salmon planted into Minter Creek			
	LV	RV	Total	Ad-An	An-RV	D-RV	Total
Area 10 / Area 11, 12	2.0	1.1	1.3	0.2	0.5	0.7	0.
Area 10, 11 / Area 12	14.0	5.2	6.5	3.0	3.8	7.0	4.4

10 and 11 than Ad-An and An-RV fish (Table III), but sportsmen caught them in area 10 during every month from March through August when very few Ad-An and An-RV fish were reported (Table II). During the Bush Point reef-net fishing in July and August 1953 the only Minter Creek mark recovered, prior to arrival of salmon returning to Puget Sound from the Pacific Ocean, was a single D-RV fish (Fig. 9).

No sportsmen returns for D-RV-marked salmon came from northern waters of Puget Sound in 1952 whereas a considerable number were reported in 1953.

MIGRATION AND DISTRIBUTION IN PACIFIC OCEAN IN 1953

Distribution in the Pacific Ocean was studied mainly through analysis of marked salmon recovered from ocean troll fisheries. Since coho salmon of less than 50 cm in fork length could not be retained by the troll fishermen in United States waters, there were no returns from this fishery until June 21, 1953. The size limit did not apply to British Columbia trollers. Lengths of Lake Washington fish recovered in the Washington troll fishery, however, indicated that all salmon probably did not become large enough to be retained by the fishermen until late July or August (Fig. 4).

Marked fish recovered are usually weighted by fishing effort when such data are used to indicate migration or relative abundance. Establishing fishing effort by area in the troll fisheries is very difficult due to the great mobility of the fleets. However, the consistency between total catch, catch by port of landing, and recovery of marks by month (Fig. 5) would indicate that the Washington trollers were fishing either the same population of salmon throughout most of the season, or fishing different populations with equal success. Only LV- and RV-marked salmon are shown in Fig. 5 as the pattern of mark recoveries for Minter Creek groups was almost identical. Such curves as shown in Fig. 5 could also be produced by having the same relative numbers of marked and unmarked salmon either entering or leaving the population being exploited off the Washington coast.

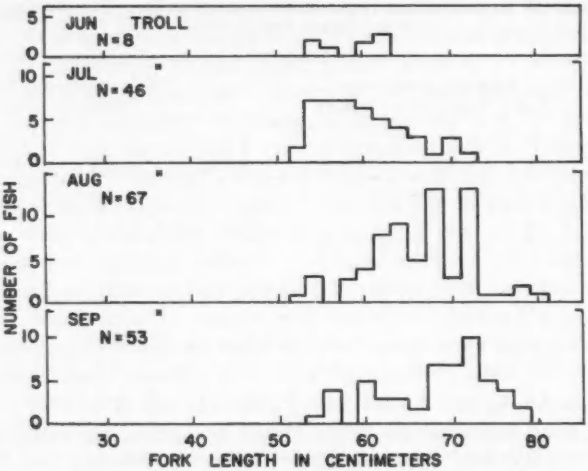

FIG. 4. Length of LV- and RV-marked coho salmon recovered in the Washington troll fishery and in the Puget Sound sport fishery in 1953.

A large number of both Lake Washington and Minter Creek salmon engaged in what I feel was a feeding migration off the Washington coast. This is indicated by plotting the marked salmon recovered in 2-week periods by area. Recovery of salmon from the Lake Washington drainage is shown in Fig. 6 and from Minter Creek in Fig. 7. The salmon were concentrated in the Swiftsure–Cape Flattery area in early July, moved southward to the Destruction Island–Grays Harbor area by late August, and finally migrated back to Cape Flattery area during September. Undoubtedly some of the LV and RV marks taken in the Grays Harbor area in late August and September were salmon with a duplicate mark planted at the Little White Salmon hatchery (Fig. 2; see Allen, 1965, for discussion of magnitude of error).

A second behavior is shown by Minter Creek marks recovered in June in the Oregon troll fishery (Fig. 8). These salmon that were in the Columbia River–Newport, Oregon, area by mid June moved north during late August and September and presumably contributed to the catch off Washington at that time. Lake Washington fish could not be followed off Oregon because single-fin marks found in the Oregon troll fishery were not recorded. However, analysis of LV and RV marks in the Oregon fishery would have been difficult because salmon bearing a duplicate mark released at Little White Salmon (Fig. 2) migrated mainly south from the mouth of the Columbia River, contributing heavily to the California troll fishery (see Allen, 1965, for discussion).

Another path of migration was shown by salmon moving northward from Juan de Fuca Strait. Details of this migration could not be followed due to the

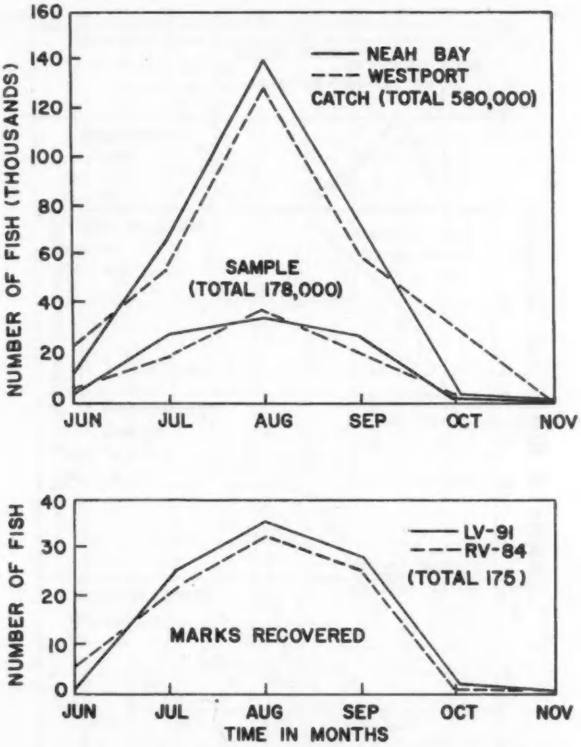

FIG. 5. Estimated number of coho salmon landed and number of salmon sampled by month in 1953 at Neah Bay and Westport, and total number of LV- and RV-marked coho salmon recovered in the sample each month.

relatively small number of fish recovered in the sampling, and insufficient data on specific area of recovery. Coho salmon migrating northward could have been scattered widely out to sea in the North Pacific Ocean and Gulf of Alaska (International North Pacific Fisheries Commission, 1957). Recoveries of marked salmon by month in the troll fishery off the west coast of Vancouver Island are listed in Table V. The recoveries are compared directly by month. The number of salmon examined each month by Canadian biologists was kept fairly constant in contrast with the Washington and Oregon sampling, which varied directly with the landings. These data showed Minter Creek and Lake Washington salmon relatively scarce off the west coast of Vancouver Island during June and July. During the last 2 weeks of August the recovery of both Minter Creek and Lake Washington salmon increased markedly (Table VI). These salmon presumably migrated into the British Columbia area from the north because few marked salmon were in the Swiftsure–Cape Flattery area

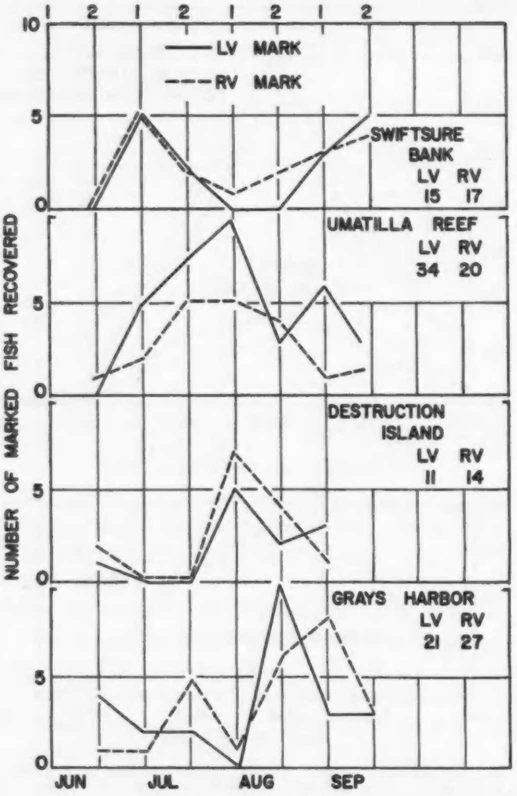

FIG. 6. Recovery during first half (1) and second half (2) of month by area of LV- and RV-marked coho salmon off Washington coast in 1953.

during August (Fig. 6 and 7). It was possible but unlikely that the troll fishery was avoided by northward-migrating salmon in the Swiftsure–Cape Flattery area in August.

Superimposed upon the broad outlines of the three major migrations described above were differences associated with each group. The most interesting difference was noted for the D-RV group from Minter Creek. As previously mentioned, this group had been planted 2 months later than the other Minter Creek fish and had reflected this later time of release by a lag in average time of arrival at Bush Point (Fig. 3). Continuance of this lag in migration would account for the highest number of recoveries of the D-RV marks off the west coast of Vancouver Island occurring in July (Table V). It would also explain the D-RV mark predominating in mid season (late July–August) in the Cape Flattery–Swiftsure Bank area (Fig. 7), and the later appearance in the season

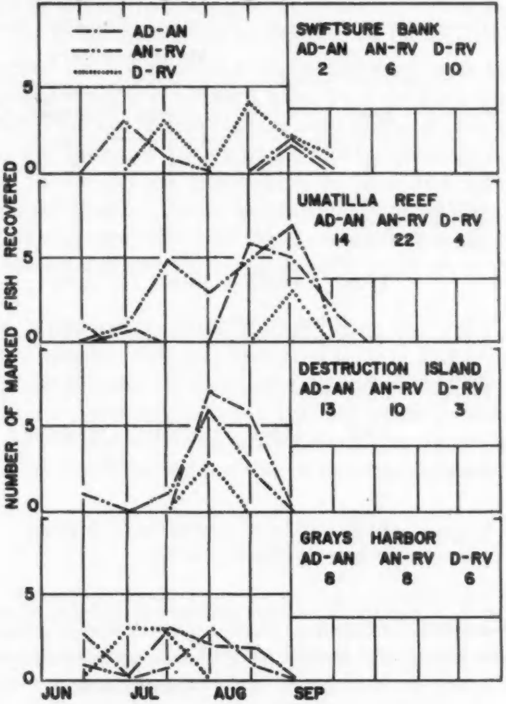

FIG. 7. Recovery during first half (1) and second half (2) of month by area of Ad-An, An-RV, and D-RV-marked coho salmon off Washington coast in 1953.

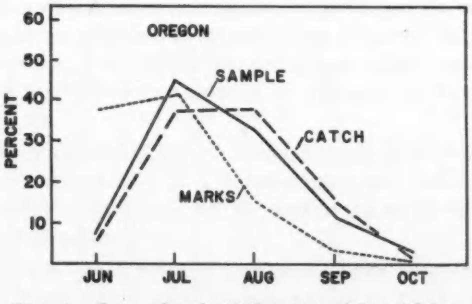

FIG. 8. Comparison in 1953 commercial troll fishery off Oregon of estimated landings (catch), number of salmon in landings examined for marks (sample), and number of marked 1950 brood coho salmon from Minter Creek (marks) as monthly percentage of season's total number.

TABLE V. Number of fin-marked coho salmon recovered in sampling of coho salmon troll fisheries off the west coast of Vancouver Island, Oregon coast, and California coast in 1953.

		Month of recovery					
Areas of recovery	Mark	June	July	August	September	October	Total
West coast of Vancouver Island	LV	1	5	12	5	0	23
	RV	1	3	5	2	0	11
	Ad-An	1	1	4	1	0	7
	An-RV	0	0	1	2	0	3
	D-RV	0	3	2	1	0	6
Number salmon examined		7,929	10,993	10,657	7,591		
Oregon coast	LV		not recorded				
	RV		not recorded				
	Ad-An	2	1	1	0	0	4
	An-RV	6	6	1	1	0	14
	D-RV	1	3	2	0	0	6
Number salmon examined		5,382	33,014	24,001	8,146		
California coast	An-RV	0	0	1	0	0	1
Number salmon examined		21,855	22,328	10,667	7,890		

TABLE VI. Number of fin-marked coho salmon recovered in sampling of catch in troll fishery off west coast of Vancouver Island in 1953, by 2-week periods.

	Period of recovery							
	June	July		August		September		
	16–30	1–15	16–31	1–15	16–31	1–15	16–30	Total
Lake Washington								
LV	1	2	3	2	10	4	1	23
RV	1	1	2	3	2	2	0	11
Total:	2	3	5	5	12	6	1	34
Minter Creek								
Ad-An	1	0	1	2	2	1	0	7
An-RV	0	0	0	0	1	2	0	3
D-RV	0	0	3	0	2	1	0	6
Total:	1	0	4	2	5	4	0	16
Lake Washington and Minter Creek	3	3	9	7	17	10	1	40
Number of salmon examined during month	7,929	10,993		10,657		7,591		37,170

of D-RV marks in the Oregon troll fishery (Table V). These data indicate not only continuance of the lag in average time of arrival to various areas but also a tendency for the D-RV group not to migrate as far as other groups.

Salmon marked An-RV showed a slight difference in behavior from other groups by migrating mainly southward from Juan de Fuca Strait and tending to migrate farther. This was inferred by the only Minter Creek salmon recovered in the California troll being an An-RV mark, and by the large number of An-RV marks appearing in the Oregon troll fishery in June and July coupled with a correspondingly small number in the Washington troll at the same time (Table V, Fig. 6). Not at variance with this thesis, was the first report by sportsmen of a Minter Creek salmon in 1952 — an An-RV mark from the head of Hood Canal.

LV salmon migrated northward from Juan De Fuca Strait in larger numbers than RV salmon. This was indicated by: (1) 23 LV and 11 RV marks recovered off the west coast of Vancouver Island (Table V), of which 10 LV and 2 RV marks were recovered during the last weeks in August (Table VI); (2) 4 LV and 1 RV marks recovered at Pelican, Alaska; and (3) 4 RV and 2 LV marks recovered from trollers fishing the 40-mile Bank (area 5, Fig. 2).

RETURN FROM PACIFIC OCEAN TO POINTS OF RELEASE IN 1953

Migration toward Juan de Fuca Strait had started off Oregon in July as indicated by pattern of recovery of An-RV marks (Table V) and of all Minter Creek marks combined (Fig. 8). Movements of all groups off the Washington coast had become a general migration northward by early September (Fig. 6 and 7). The main body of salmon returning from northern areas clearly passed off the west coast of Vancouver Island during the last 2 weeks of August and the first 2 weeks of September (Table VI). The salmon passed Cape Flattery by the end of September as indicated by the large returns of sport-caught salmon taken in the Pacific Ocean in September and none in October (Table II). Although there was no measure of sport-fishing intensity in the Cape Flattery area for these months, the fishing intensity generally was influenced by the presence or absence of catchable salmon in this area during the years of this study.

Progress of the migration through Puget Sound could be studied by recoveries in the Bush Point reef-net, in a purse-seine fishery in Admiralty Inlet, in a gillnet fishery north and west of Seattle, and in the sport fisheries in Shilshole and Elliot bays off Seattle.

Only two Minter Creek salmon and three Lake Washington salmon were recovered in the Bush Point reef-net operations in 1953 (Fig. 9). Few coho salmon were caught prior to August 31 in the reef-net because fishing was aimed at capturing pink salmon for tagging. The pink salmon were being taken in large numbers by relatively few lifts of the net; hence, fishing effort was restricted. It was felt, however, that the coho salmon caught in the reef-net were representative of the size frequency of the coho salmon passing the area at that time (Hans Jensen, personal communication). Most coho salmon feeding in the Pacific Ocean attained a length of greater than 60 cm by September, whereas very few salmon remaining within Puget Sound attained a length of greater than 58 cm (Fig. 3; Allen, 1959b). In early September relatively more salmon over 60 cm occurred in the catch of the reef-net at Bush Point (Fig. 9). This indicated that the first substantial arrival of unmarked salmon from the Pacific Ocean was occurring at that time.

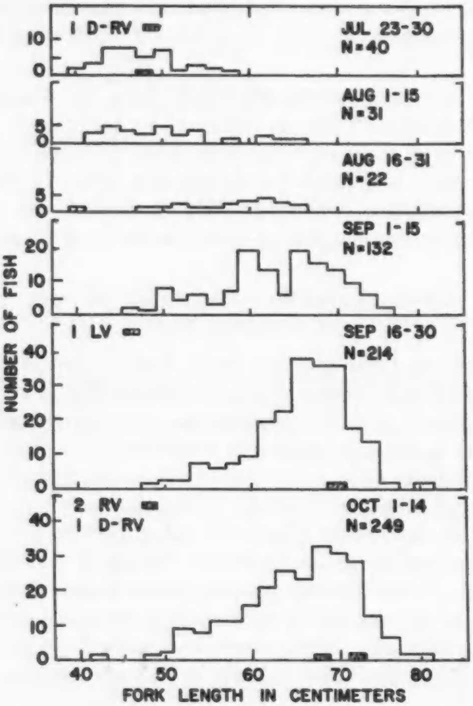

FIG. 9. Length of coho salmon taken in reef-net fishing at Bush Point, Puget Sound, in 1953, and length of marked coho salmon captured (D-RV marked fish taken in October not measured).

The main body of Lake Washington and Minter Creek salmon had not entered Puget Sound by early September. This was indicated by the relatively few marks obtained from a sample of salmon taken by purse-seiners fishing on both sides of Puget Sound in the Admiralty Inlet area (Table VII). By late September, however, both Minter Creek and Lake Washington salmon were recovered in large numbers from the gillnet fishery operating in the Seattle

TABLE VII. Number of fin-marked coho salmon recovered in sampling of catch of Admiralty Inlet purse-seine and Seattle area gillnet fisheries in 1953.

Area of recovery and fishery	Period of sampling	Mark							Number of salmon examined
		LV	RV	Total	Ad-An	An-RV	D-RV	Total	
Admiralty Inlet (purse seine)	Aug. 24–31	0	1	1	1	0	0	1	439
	Oct. 12–15	11	8	19	0	1	3	4	1,880
	Total:	11	9	20	1	1	3	5	2,319
Seattle area (gill net)	Sept. 27 to Oct. 1	6	4	10	8	2	1	11	1,947
	Oct. 4–9	25	26	51	11	3	3	17	4,472
	Oct. 11–19	8	2	10	8	2	1	11	824
	Total:	39	32	71	27	7	5	39	7,233

area (Table VII). Large numbers of both Minter Creek and Lake Washington salmon were recovered from Admiralty Inlet to Seattle from October 4 to 19 (Table VII). During October and early November, 4 LV and 2 RV marks were reported from sport boats fishing out of Shilshole Bay, which is the entrance to the Lake Washington drainage. At the same time no recoveries were made from sport fishermen landing salmon at boat houses located on Elliot Bay which is the mouth of the Green River drainage. No recoveries were reported from the sport fishery after November 11 (Table II). Thus, the main body of Lake Washington salmon had apparently passed into fresh water by the end of October.

Return of LV salmon to the University of Washington ponds began in late November, with peak returns in late December and early January. The run was not completed until late February. RV fish were first captured at the Issaquah Hatchery rack in mid October, with the peak of the run occurring in early December. The Minter Creek salmon first appeared at the hatchery in late September, arriving in peak numbers in late November. The run ended in early January. Details of freshwater movements can be found in Donaldson and Allen (1958), Allen (1959a), and Salo and Bayliff (1958).

The four Minter Creek marks taken in the Admiralty Inlet fishery in the period of October 12–15 are of special interest (Table VII). Three of these four salmon were marked D-RV. Ordinarily very little significance would be attached to such small numbers. These data, however, could be interpreted as a later average time of migration for the D-RV group similar to the interpretations made for the pattern of D-RV marks in other fisheries. That such inferences were indicating real effects was given strong support by Salo and Noble (MS, 1954) who reported that only 29% of the D-RV group had returned to the

Minter Creek rack by November 15 at which time roughly half of the total An-RV and Ad-An groups had been recovered.

RATE OF MIGRATION

Large numbers of Minter Creek and Lake Washington salmon were recovered off the central Washington coast in early September (Fig. 6 and 7). Peak recovery of marked salmon in the Seattle area gillnet fishery was during the first 2 weeks of October (Table VII). About 3–4 weeks elapsed from the time the bulk of the salmon moved from the central Washington coast to middle Puget Sound. This distance is about 150 nautical miles. The salmon, therefore, were moving at an average rate of 5–7 nautical miles per day during their migration from the Pacific Ocean to Puget Sound.

SUMMARY

Salmon planted into Minter Creek (southern Puget Sound) remained within Puget Sound to a greater degree than salmon planted into the Lake Washington drainage (middle Puget Sound). Of salmon remaining within Puget Sound as feeding adults, those planted at Minter Creek were more equally distributed throughout Puget Sound in contrast to those planted into the Lake Washington drainage which were rarely recovered from southern areas.

Three major migrations could be discerned for marked salmon leaving Puget Sound and entering the Pacific Ocean. One group of fish was found off the Oregon coast in June 1953, migrating northward with progression of the season. A second group of fish found off Cape Flattery in June moved south during the summer off the Washington coast before migrating northward back to Puget Sound. A third group of fish went north from Juan de Fuca Strait. Details of this northward migration could not be followed, although the main body of salmon migrated into waters off Vancouver Island in late August, presumably from the north.

Differences in the place and time of migration in Puget Sound and the Pacific Ocean between groups of salmon were related to differences in age of planting, method of planting, and distance from salt water of planting. Of three groups of salmon planted in southern Puget Sound, the control group (An-RV mark) tended to migrate farther south in the Pacific Ocean than the other two groups. Of two groups of salmon planted in middle Puget Sound drainage, salmon planted relatively near salt water (LV mark) migrated northward on reaching the Pacific Ocean in relatively larger numbers than did salmon planted farther inland (RV mark). The RV-marked salmon showed a slight delay in reaching salt water and in leaving Puget Sound as compared to the LV-marked group.

Salmon planted into southern Puget Sound which had 2 additional months of rearing as compared to all other groups (D-RV mark), were delayed in leaving Puget Sound in 1952, in entering and leaving the troll fisheries in the Pacific Ocean, and in re-entering Puget Sound. This later average time of migration indicated by analysis of marked fish recovered in ocean fisheries was substantiated by a later average time of arrival recorded at the Minter Creek hatchery where the salmon were released.

ACKNOWLEDGMENTS

Every assistance in providing records of marked fish was given by personnel of the Pacific Marine Fisheries Commission, the Fisheries Research Board of Canada, The Washington Department of Fisheries, the Oregon Fish Commission, the California Department of Fish and Game, and the United States Fish and Wildlife Service. Such cooperation is gratefully acknowledged. I wish to thank Dr Richard Ridenhour for valuable suggestions made in reviewing this manuscript.

Much information necessary for this analysis was obtained from Progress Reports of research conducted by the State of Washington Department of Fisheries. These reports are mimeographed and given limited distribution among research and other divisions of the department. Generally these reports are available for reference; however, the department reserves the right to withhold data on research which is incomplete or still in progress (Donald R. Johnson, 1954, Research Director, Washington State Department of Fisheries, personal communication).

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Taste Panel Assessment of Cod Fillets After Single and Double Freezing[1,2]

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ABSTRACT

Taste panel assessment is reported for Atlantic cod (*Gadus morhua*) frozen once and twice, and stored at −23 C. The fish were bled, gutted, and iced immediately after capture, and frozen as fillets or dressed fish before, during, and after rigor mortis. Dressed fish were thawed later in recirculated tap water, then processed, refrozen, and stored as fillets. The procedure was conducted three times (June 27, July 19, July 30) during the inshore trap fishery and twice (March and October) during the offshore Grand Bank fishery.

For trap-caught landings, the quality of the stored fillets frozen once depended upon the season of catch; for Grand Bank cod, upon the time and place of catching. Trap fish, feeding lightly, caught in cold water in June, were of best quality, grades equalling 70 and over after 20 weeks storage. Those caught on July 30 while feeding heavily in warm water were second; the July 19 fish, obtained under nearly similar conditions, were not significantly poorer than those taken on July 30 and were still acceptable. March landings of Grand Bank fish, once frozen, scored over 70 after 20 weeks storage; October landings only 40 (borderline quality) after 30 weeks. Physiochemical and chemical assessment confirmed the poor quality of the latter.

Thawing, refreezing, and storage of June trap fish longer than a few weeks resulted in poor but still acceptable samples. The stored product was soon similar in quality to samples prepared from July landings. In contrast, twice-frozen samples prepared from the March landings from the Grand Bank continued to score high, 70 after 28 weeks storage. Refrozen October samples from the Grand Bank yielded much lower scores, similar to those given the once-frozen samples of the same catch. Thus, in general, an acceptable or better twice-frozen product was obtained by starting with material well handled and quickly chilled, from either the inshore or offshore fishery.

INTRODUCTION

The newfoundland frozen fish industry is lacking in a steady supply of raw material. To remedy the situation important segments of the industry might wish to add to present landings of unfrozen fish such quantities of eviscerated,

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[1] Received for publication August 20, 1965.

[2] This is the eighth paper in a series on quality of cod. The previous seven are listed in Appendix I. The tenth and eleventh appeared in 1965 (J. Fish. Res. Bd. Canada, **22**: 411–420 and 783–790. The ninth is in preparation.

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eviscerated and headed, or whole groundfish as may be taken economically in coastal or in offshore waters and frozen between plates or by immersion methods aboard suitably equipped vessels. The frozen fish would provide raw material for thawing, processing, and refreezing in the periods when iced or uniced fish are in short supply or are not available at the plants. Already one processor has taken steps to supplement landings from conventional iced-fish trawlers with block-frozen fish prepared on stern ramp trawlers equipped for freezing at sea (Walsh, 1965). This paper reports on the quality (as assessed by an analytical taste panel — baking procedure) in storage of the frozen and refrozen products from Atlantic cod trapped inshore or trawled on the Grand Bank.

In earlier papers Dyer et al. (1962) and MacCallum et al. (1964) reported that refrozen fillets from late-July trap landings deteriorated rapidly during the first 2 months in frozen storage at either −18 C or −23 C, and that higher values for thaw-drip and free fatty acids were found in refrozen fillets from late-July trap landings than in similarly handled fillets of fall-caught inshore cod. The present work supplements the earlier studies. But more important, it examines once- and twice-frozen trap-caught cod for seasonal variations and for the effect of initial freezing before and in rigor mortis. It also presents the results of taste panel and chemical evaluations of the quality of Grand Bank cod exposed to similar freezing, thawing, refreezing, and storage cycles.

MATERIALS AND METHODS

Pre- and In-Rigor Trap-Caught Cod and Trawler-Caught Controls

Cod were trapped near St. John's on June 27, 1962, boated from surface water at 4 C, gutted, iced at the trap, landed within about 1 hour and reiced. The fish, 2–3 lb when gutted, were then divided into two lots (Fig. 1) for freezing, one before and the other in rigor.

Another lot of fish (3–4 lb gutted weight), obtained on the southeast edge of the Grand Bank on June 25, 1962, was iced aboard a trawler for 2–3 days and upon landing was filleted and frozen (post rigor) as a once-frozen control. Processing, freezing, and storage procedures for the Grand Bank fish were as described below for the once-frozen trap fish controls (fillets).

For freezing before rigor, some of the trap fish were washed, filleted, skinned, packaged in 1-lb cartons, wrapped in waxed paper, and frozen to −23 C (in 1¼ hr) in a horizontal-plate freezer within 4 hr of capture. These samples, to be used as once-frozen fillet controls, were packed in commercial cardboard cartons which were sealed with masking tape and stored in an insulated box held in a commercial cold store at −23 C. Other pre-rigor fish were headed, washed, and frozen in 18-lb blocks in a vertical-plate freezer for use as once-frozen dressed[4] controls and, after thawing, in the production of twice-frozen fillets. The blocks, each measuring approximately 4 × 36 × 4½ inches, were

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[4] Beheaded and gutted but not de-finned or trimmed at the nape.

Starting material		Treatment	
Gutted, iced cod, trapped June 27, 1962 (1 hr to landing)	Pre-rigor (iced 4 hr)	Frozen fillets (controls)	
		Frozen (18-lb) blocks of headed, gutted fish stored for use as	(a) dressed controls (b) material for thawing (in 7.2 C water for 3 hr), processing and refreezing; 1 day after first freezing 12 weeks after first freezing
	In-rigor (iced 24 hr)	Frozen fillets (controls)	
		Frozen (18-lb) blocks of headed, gutted fish stored for use as	material for thawing, processing and refreezing; 1 day after first freezing 12 weeks after first freezing
Trawler-caught Grand Bank cod, gutted and iced at killing, post rigor on landing		Frozen fillets (controls)	

FIG. 1. Flow diagram showing treatment of frozen, dressed trap cod (controls), fillets of trawler-caught cod (controls) and trap fish (controls) and of other trap fish frozen twice, first as dressed fish, later as processed fillets.

frozen to −23 C in 5½ hr. Following freezing the blocks, 15 in all, were wrapped in 4-mil (.004-inch) polyethylene sheets, sealed with masking tape, and stored at −23 C under conditions similar to those for the fillets prepared from the trap-caught and Bank fish.

For freezing in-rigor, the iced trap fish were divided into two lots 24 hr after being landed, their temperature then being 0 C. One lot was processed and frozen as once-frozen fillet controls and the other as dressed fish. The latter were used, after thawing, in the production of twice-frozen fillets. Each lot was handled, frozen, and stored in the same way as the corresponding product frozen before rigor.

For refreezing (1 day after first freezing and again after 12 weeks frozen storage), segments were first separated from the frozen blocks of dressed fish in the cold store. Then the fish were removed from the store and thawed in recirculated fresh water at 7.2 C for 3 hr (MacCallum and Ellis, 1964), processed, packed, frozen, and again stored like the fillet controls.

COD TRAPPED ON THREE OCCASIONS DURING THE FISHING SEASON AND FROZEN IN RIGOR

The source of the early-season fish (24 hr in ice) was as described above. Other lots of cod were handled similarly on July 19 and 30. Each of the three lots was used to produce once-frozen fillets and block-frozen dressed fish

(Fig. 2). All fish were stored at −23 C. The dressed packs were thawed for 1¼ hr in recirculated water at 15.5 C, then immediately filleted, skinned, packed, refrozen, and returned to −23 C storage. Samples were thawed and refrozen after storage periods of 1 day and 12 weeks (early season), 3 days and 12 weeks (middle of the season) and 2 days and 16 weeks (late middle season).

Starting material	**Treatment**		
Gutted, iced (24 hr after killing) cod, trapped near the early, middle, and late-middle periods of the summer season, in-rigor at first freezing		Period (of killing) during trap season	
	Once-frozen fillets (controls)	early (June 27, 1962)	1
		middle (July 19, 1962)	2
		late middle (July 30, 1962)	3
	Once-frozen as dressed fish, thawed in water at 15.5 C, then filleted and refrozen		
	Finished fillets produced;		
	2 days after capture	early	4
	3 days after capture	late middle	5
	4 days after capture	middle	6
	12 weeks after capture	early	7
		middle	8
	16 weeks after	late middle	9

(Numbers in right-hand column refer to curves in Fig. 6.)

FIG. 2. Flow diagram showing treatment of once- and twice-frozen in-rigor fillets of trap cod obtained on three occasions during the fishing season, 1962.

TRAWLER-CAUGHT FISH

MARCH 1962 CATCH

Market size (6-lb gutted weight) Grand Bank cod were gutted and iced at sea, landed post-rigor after 4 days, prepared as fillets and frozen, or frozen as dressed fish. The latter were thawed, processed, refrozen, and again stored (Fig. 3) using the procedures followed for trap fish.

OCTOBER 1964 CATCH

Cod (2½–3½ lb gutted weight), lightly feeding on sand launce, were taken in three successive tows of 1 hr each halfway up the east edge of the Grand Bank in October 1964, and immediately gutted and iced (Fig. 4). Headed, gutted fish and fish fillets were frozen before and in rigor (20 hr in ice) in 42″ × 14″ × 4″ waxed paper cartons and in 12 oz waxed paper cartons, respectively, aboard the *A. T. Cameron*. All samples were frozen at 1⅓ psi between two metal plates. The top plate was refrigerated by forced air at a temperature of −27 C, the bottom by expanded Refrigerant-12 (average plate

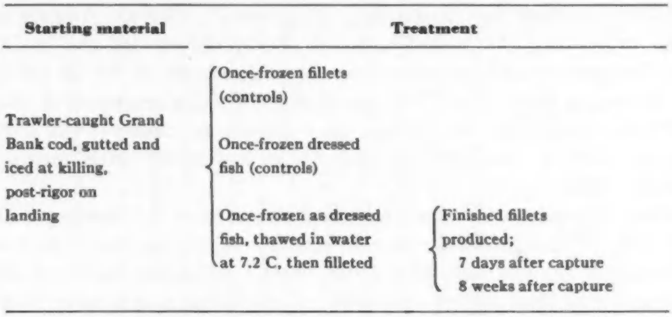
Starting material
Treatment
Trawler-caught Grand Bank cod, gutted and iced at killing, post-rigor on landing
Once-frozen fillets (controls)
Once-frozen dressed fish (controls)
Once-frozen as dressed fish, thawed in water at 7.2 C, then filleted
Finished fillets produced;
7 days after capture
8 weeks after capture

FIG. 3. Flow diagram showing treatment of once-frozen dressed and once- and twice-frozen post-rigor fillets of March (1962) Grand Bank cod.

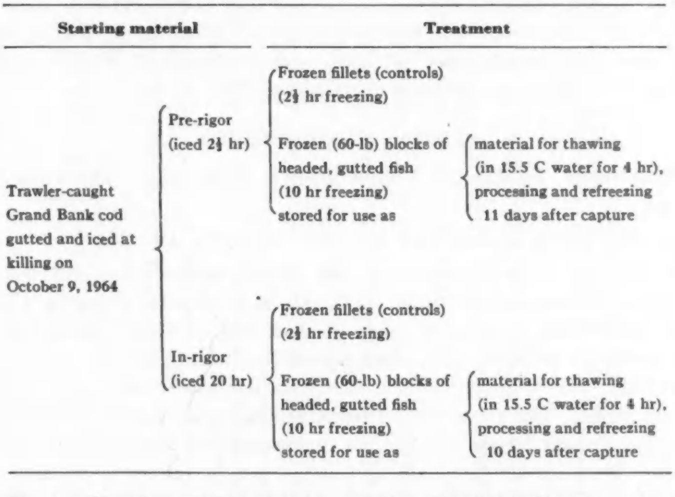
Starting material
Treatment
Trawler-caught Grand Bank cod gutted and iced at killing on October 9, 1964
Pre-rigor (iced 2½ hr)
Frozen fillets (controls) (2½ hr freezing)
Frozen (60-lb) blocks of headed, gutted fish (10 hr freezing) stored for use as
material for thawing (in 15.5 C water for 4 hr), processing and refreezing
11 days after capture
In-rigor (iced 20 hr)
Frozen fillets (controls) (2½ hr freezing)
Frozen (60-lb) blocks of headed, gutted fish (10 hr freezing) stored for use as
material for thawing (in 15.5 C water for 4 hr), processing and refreezing
10 days after capture

FIG. 4. Flow diagram showing treatment of once- and twice-frozen pre-rigor and in-rigor fillets of October (1964) Grand Bank cod.

temperature, –30 C). Time for freezing to –18 C at the centres of the 12-oz fillet cartons and the large blocks was about 2½ and 10 hr, respectively. The 12-oz cartons were wrapped in Saran. The large blocks were held unwrapped for 10 and 11 days, then thawed ashore (in 4 hr) in recirculated fresh water at 15.5 C. These fish were then processed and frozen in 12-oz cartons in 1 hr. All once- and twice-frozen fish were stored at about –23 C.

TASTE PANEL TESTING

Taste panels were conducted on all the processed products and on the once-frozen dressed fish. Evaluation followed the procedures of Dyer and

Dyer (1949) modified for frozen fish (Dyer et al., 1964a). A scale of 0–100 was used in scoring the baked samples. Forty represents the dividing line between acceptable and unacceptable samples; scores of 80–90 are reserved for fish of best quality. The 1962 panel recorded the sequence of changes in texture from tender to very tough (and fibrous or chewy); the 1964 panel recorded the above changes and also those associated with mealy texture (Idler et al., 1964).

Statistical analyses of data followed the system of Snedecor (1956, p. 136–138, 398). The significance in the variance of a slope from the horizontal was determined by the null hypothesis test. Confidence limits of the mean lines or regression lines and of the mean of individual scores were determined.

OTHER TESTS

Supporting analyses were performed according to the following procedures: thaw-drip determination, Dyer et al. (1962); chloroform-methanol phase separation procedure for the determination of total lipid, Bligh and Dyer (1959). Free fatty acids were titrated against alcoholic NaOH and results calculated for a fatty acid molecular weight of 300.

RESULTS AND DISCUSSION

PRE-RIGOR AND IN-RIGOR TRAP-CAUGHT COD AND TRAWLER-CAUGHT CONTROLS

QUALITY AFTER FREEZING ONCE AND STORING AT −23 C

The quality of the dressed trap fish frozen before rigor and that of the fillets frozen before and in rigor was high shortly after freezing (Table I).

The quality of samples of each lot handled as above, and that of the trawler controls, as shown by the regression lines (Fig. 5), depreciated in frozen storage but average overall acceptability scores were still good after 22 or more weeks. The three lots of samples from trap fish had essentially the same quality during frozen storage, as the slopes and intercepts of the curves are similar.

The quality of the trawler controls (Table I) was not significantly poorer (Fig. 5, A–D) than that of the trap fish samples after similar periods of storage. Scores given the Bank fillets were similar to those reported earlier for freshly frozen fillets of cod taken offshore between August and December, gutted and iced and landed after rigor (Dyer et al., 1962).

The quality of the dressed and filleted trap fish frozen before rigor was high in comparison with that of trap fish handled similarly in 1964 (initial scores of 60 and 75 for June 19 and July 8 catches, respectively (MacCallum, unpublished data). The 1964 samples were downgraded due to a mealy texture.

COMPARISON OF QUALITY AFTER FREEZING ONCE AND FREEZING TWICE FOLLOWED BY STORAGE AT −23 C

Within 1 week of capture, the twice-frozen samples (Table II) frozen before and in rigor did not differ significantly in quality nor were the scores

Table I. Average taste panel scores[a] during storage at −23 C for dressed and filleted cod from fish taken in the traps on June 27, 1962, and frozen once following processing.[b]

Weeks in storage after capture	Dressed cod frozen before rigor			Fillets frozen before rigor			Fillets frozen in rigor		
	Texture	Taste	Overall acceptability	Texture	Taste	Overall acceptability	Texture	Taste	Overall acceptability
1	84±2.4(21)	74±4.3(21)	81±2.9(21)	92±2.7(20)	81±6.1(20)	87±3.5(20)	90±2.2(20)	78±4.4(20)	87±2.1(20)
3				86±3.8(18)	77±3.8(18)	84±3.2(18)	85±3.8(17)	81±4.2(17)	85±2.9(17)
8	92±2.8(14)	86±3.9(14)	90±2.3(14)	90±4.1(15)	83±5.2(15)	86±4.4(15)	90±2.4(15)	79±3.7(15)	86±2.3(15)
12	73±6.1(15)	71±6.6(15)	73±4.6(15)	83±3.5(15)	78±6.0(15)	82±4.3(15)	80±3.5(15)	75±4.9(15)	80±5.8(15)
16				79±5.1(15)	64±3.9(15)	73±4.5(15)	86±8.6(15)	69±6.5(15)	83±3.1(15)
22	80±2.4(15)	67±2.8(15)	75±2.8(15)	73±3.3(24)	58±4.4(24)	68±2.6(24)	74±3.3(27)	63±3.7(27)	70±3.1(27)
28	82±2.8(13)	69±6.2(13)	77±3.7(13)	84±1.7(21)	69±3.3(21)	79±0.7(21)			

[a] Average scores are followed by the standard error of the mean and by the number of judgments (in parentheses).

[b] Fillets from Grand Bank cod obtained on June 25, 1962, frozen after rigor (2–3 days in ice) were given the following overall acceptability scores when examined along with the trap fish at 1, 8, 16 weeks, respectively: 81±2.7(19); 76±5.4(15); 66±5.4(15).

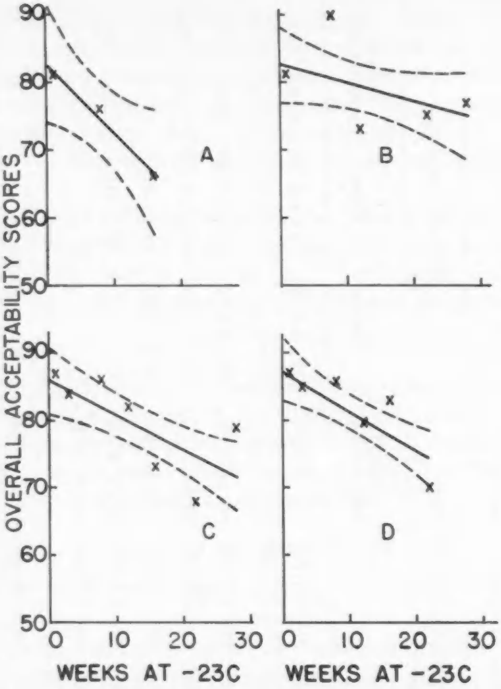

FIG. 5. The regression lines (solid) and 95% confidence limits (dashed) of the regression lines of taste panel overall acceptability scores on the time in storage at –23 C of dressed fish and fillets of trap-caught cod taken off St. John's, Nfld., on June 27, 1962, and of fillets of cod taken on the Grand Bank. Average taste panel scores are denoted by X. All fish were frozen once, after being bled, eviscerated, and iced at capture and after filleting. A — Grand Bank control fillets, frozen after rigor; B — dressed trap cod, frozen before rigor; C — fillets of trap cod, frozen before rigor; D — fillets of trap cod, frozen in rigor.

significantly different from those given the once-frozen controls (Comparison, Tables I and II).

Sixteen and 28 weeks after capture the twice-frozen samples processed as above were of much lower quality (average overall acceptability score about 50). At the former time, fillets frozen once (before rigor) were better ($P = 0.05$) than fish frozen twice, whether initially frozen before or in rigor. The once-frozen fillets prepared in rigor were better still. At the latter time, the fillets frozen once before rigor (fillets frozen in rigor not tested) and the dressed fish frozen once before rigor were better ($P < 0.05$) than samples from the two lots that were frozen twice.

Initially, the samples processed and refrozen 12 weeks after capture were also of the same quality as once-frozen samplings of the same catch (Table II).

Table II. Average taste panel scores[a] during storage at −23 C for refrozen fillets from cod taken in the traps on June 27, 1962, gutted, iced, frozen in blocks, stored, and thawed in water at 7.2 C.

Total weeks in storage after capture	Storage time (prior to thawing, processing, and refreezing) for dressed fish frozen before rigor						Storage time (prior to thawing, processing, and refreezing) for dressed fish frozen during rigor					
	1 day			12 weeks			1 day			12 weeks		
	Texture	Taste	Overall acceptability	Texture	Taste	Overall acceptability	Texture	Taste	Overall acceptability	Texture	Taste	Overall acceptability
1	80±4.2(20)	81±3.9(20)	80±3.3(20)				78±4.2(19)	79±2.8(19)	79±3.7(19)			
3	73±4.4(18)	70±3.7(18)	72±3.7(18)				82±4.2(17)	72±5.2(17)	79±4.0(17)			
8	87±3.7(15)	71±5.9(15)	81±3.7(15)				83±4.8(15)	77±3.7(15)	82±4.3(15)			
12				74±5.4(15)	67±2.3(15)	71±5.1(15)				72±4.6(15)	70±5.1(15)	71±4.4(15)
15				61±7.8(15)	58±4.7(15)	60±8.5(15)				57±5.8(15)	51±5.2(15)	55±5.4(15)
16	47±6.3(15)	45±4.7(15)	45±5.3(15)				55±5.0(15)	51±3.2(15)	53±4.7(15)			
24				70±3.9(23)	59±3.7(23)	66±3.4(23)				56±4.0(27)	60±3.4(27)	60±3.0(27)
28	46±4.3(19)	49±3.9(19)	44±2.8(19)				66±5.4(16)	49±7.4(16)	57±7.2(16)			
30				49±4.5(13)	50±4.9(13)	47±5.0(13)				47±2.2(16)	51±3.2(16)	47±2.2(16)

[a] Scores are followed by the standard error of the mean and by the number of judgments (in parentheses).

These twice-frozen samples deteriorated at approximately the same rate after preparation as those refrozen within the first week of capture.

Cod Trapped on Three Occasions During the Fishing Season and Frozen in Rigor

ONCE-FROZEN FILLETS

All samplings prepared at the middle (July 19) and late-middle (July 30) periods of the trap season were poorer (P = 0.05) than those of the early season (June 27) lot immediately after freezing (Fig. 6) and, as assessed from

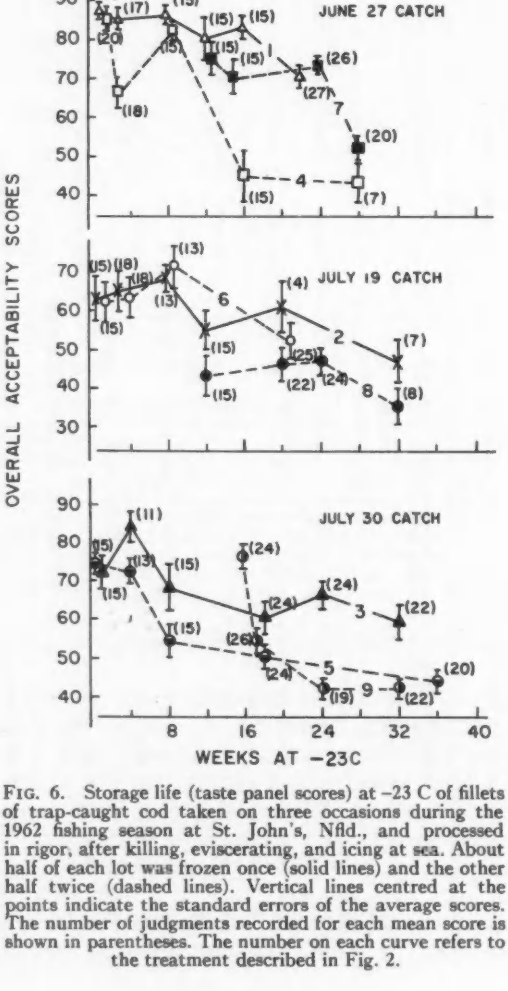

FIG. 6. Storage life (taste panel scores) at −23 C of fillets of trap-caught cod taken on three occasions during the 1962 fishing season at St. John's, Nfld., and processed in rigor, after killing, eviscerating, and icing at sea. About half of each lot was frozen once (solid lines) and the other half twice (dashed lines). Vertical lines centred at the points indicate the standard errors of the average scores. The number of judgments recorded for each mean score is shown in parentheses. The number on each curve refers to the treatment described in Fig. 2.

the confidence limits of the regression lines (Fig. 7), after extended storage. There was a significant and equal falling off in the regression lines for fish of the three lots. The late-middle season samples were not significantly better than those of the mid-season lot.

Unpublished work at the Technological Unit shows flesh glycogen of trap-caught cod at killing to increase to 0.36 from 0.07% and drip losses in unfrozen samples filleted pre rigor to increase to 3.9% from 1.5 (values after 24 hr fillet storage at 4 C) during the June–August fishing period. The *p*H level of eviscerated iced fish was found to range from 6.75 (June 19 samples uniced 9 hr, then iced for 16 hr) to 6.32 (August 10 samples uniced 5 hr, then iced for 24 hr). The same work, dealing with August catches filleted during rigor, shows a clear trend towards higher free drip values with decreasing *p*H.

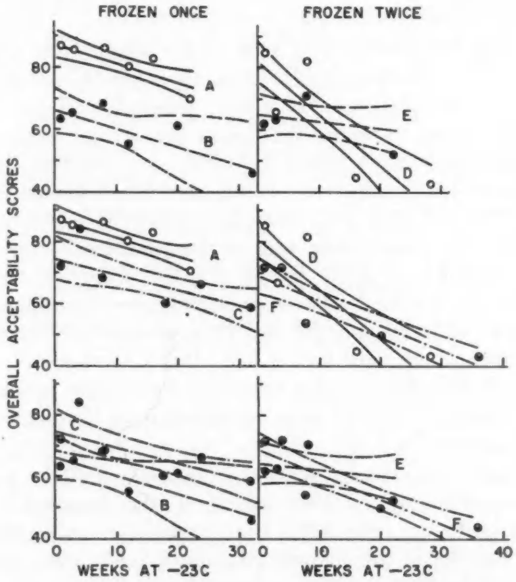

FIG. 7. The regression lines and 95% confidence limits of the regression lines of taste panel overall acceptability scores on the time in storage at −23 C of once- and twice-frozen fillets of trap-caught cod taken on three occasions during the 1962 fishing season at St. John's, Nfld., and processed in rigor, after killing, eviscerating, and icing at sea. Discontinuities in the hyperbolic pattern of the curves showing the confidence limits, e.g. curve B, result from unequal numbers at the various points. A and D (solid lines), B and E (dashed lines), and C and F (dashed and dots) —June 27, July 19, and July 30 catches, respectively. Refreezing 1 day (June 27 lot), 3 days (July 19 lot), and 2 days (July 30 lot) following capture. ○, ●, ◒ — average scores given June 27, July 19, and July 30 samples, respectively.

Thus, we have evidence of susceptibility of late July and early August trap fish to the development of large amounts of drip and low post mortem *p*H. As shown by Finn (1933–34), the latter can account for increased denaturation (in fish muscle juice). Indeed, the middle and late July samplings were tougher immediately after freezing and after frozen storage than the samples prepared in June.

The scores given the July samples, although low in comparison with June samplings, are still considerably higher than the score of 50 given stored dressed trap cod taken at a comparable period in July 1961 (Dyer et al., 1962). There are no experimental data to explain this difference.

TWICE-FROZEN FILLETS

Immediately upon refreezing, grades were generally similar to those for the once-frozen fillets (Fig. 6). Samples thawed and refrozen after 12 weeks (middle-season lot) are exceptions.

But after very short periods of storage the single June and the two July catches were of equal quality (Fig. 7). Thus, there was more rapid deterioration in the quality of the fish (June 27 catch) given the highest score initially. Samples showing the next greatest drop in ratings during refrozen storage were those given the next highest scores initially, viz. the July 30 samplings. A corollary to the above is that samples in general of lowest quality initially (viz. the July 19 catch) were not reduced in quality when refrozen and stored at −23 C. Scores for refrozen samplings of the June catch started at about 85 and decreased to about 45 at 16 weeks (Fig. 6). The refrozen samples from fish taken on July 19 (curve 6) and on July 30 (curve 5) dropped in score from 62 to 52 and from 72 to 50 after 20 and 18 weeks storage, respectively. Thus, although the scores given once-frozen fish after a storage period of 22 weeks were higher for those taken in June than for those taken in July (see above), differences due to season of catch were not apparent in the twice-frozen samples held for comparable periods.

The scores given the two twice-frozen lots prepared in July were much higher than reported for twice-frozen cod taken at a comparable period in 1961 (Dyer et al., 1962). In the case of the 1961 samples, considerable deterioration in quality was observed in the refrozen product 8 weeks after capture. Average scores (Fig. 6) for the 1962 samples, refrozen immediately or after a period of storage as dressed fish, show such a deterioration when held in refrozen storage more than 8 and 12 weeks, respectively (both June 27 and July 19 catches), and after much shorter periods (4 and 0 weeks, respectively, July 30 catch). It would appear from this and from the summary of results (Fig. 8) that a catcher-processor would have to be selective to produce and market high quality twice-frozen trap cod. Storage of refrozen material would have to be short unless the high initial quality of fish taken at the start of the trap season were to be forfeited. However, cod of poorer initial quality, e.g. samples in the category of the July 19 catch, may be refrozen and stored at −23 C for almost as long as once-frozen fish without materially altering the quality.

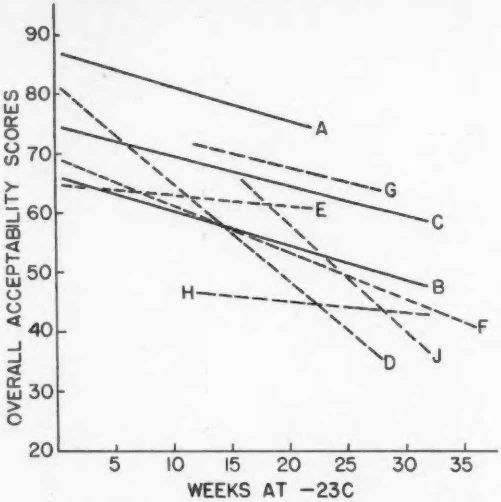

FIG. 8. A summary showing the regression lines of taste panel overall acceptability scores on the time in storage at −23 C of fillets of trap-caught cod taken on three occasions during the 1962 fishing season at St. John's, Nfld., and, after killing, eviscerating, and icing at sea, processed in rigor and frozen once (solid lines) or twice (dashed lines), first as dressed fish and later as fillets. A — June 27 catch; D — June 27 catch, final freezing June 29; G — June 27 catch, final freezing September 17; B — July 19 catch; E — July 19 catch, final freezing July 23; H — July 19 catch, final freezing October 15; C — July 30 catch; F — July 30 catch, final freezing August 2; J — July 30 catch, final freezing November 26.

Analyses of the record sheets (taste panel) provide the following summary of comments. Twice-frozen fillets of the three catching periods were downgraded (after 28 weeks at −23 C) on the basis of increasing toughness, fibrosity, and chewiness which in some instances had developed to a marked degree. The samples were tasteless for the most part while some samplings showed evidence of slightly stale, stale, and sour flavours. However, overall acceptability scores were no poorer than those given once-frozen trap fish fillets (scores after 4–6 months frozen storage in the plants ranged from 25 to 67, with an average score of 41) prepared commercially at widely separated points in the province in the same year (Dyer et al., 1962, unpublished data). It is apparent that the former samples were of marketable quality 28 weeks after capture although they did not have much reserve quality for any further deterioration which might occur during marketing and distribution.

TRAWLER-CAUGHT FISH

MARCH 1962 CATCH

All once-frozen fillets, dressed fish, and the refrozen fillets prepared at 7 days were of very good quality initially (Fig. 9).

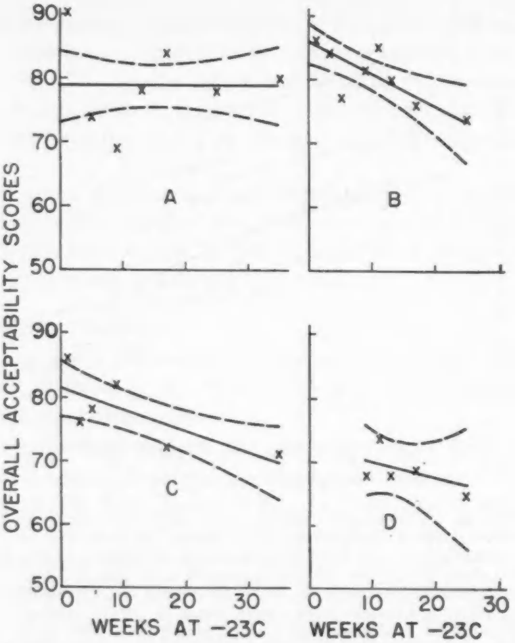

FIG. 9. The regression lines (solid) and 95% confidence limits (dashed) of the regression lines of taste panel overall acceptability scores on the time in storage at −23 C of dressed fish and fillets of Grand Bank cod taken in March 1962 and processed after rigor, after killing, eviscerating, and icing at sea. Average taste panel scores are denoted by X. A — dressed controls, frozen once; B — fillet controls, frozen once; C — refrozen fillets of dressed fish, final processing 7 days after capture; D — refrozen fillets of dressed fish, final processing 8 weks after capture.

Although the quality of the once-frozen fillet control fell during storage for 25 weeks at −23 C, and that of the dressed control was fairly constant, there was no statistical difference in the quality of the two samples during the period.

The similarity observed initially in the average scores (Fig. 9) of once- and twice-frozen samples prepared in March (Fig. 9, A–C) was maintained generally for all periods of storage with one notable exception. After 35 weeks storage, the refrozen samples were poorer ($P = 0.05$) than once-frozen dressed samples. On the other hand, even the lowest score was well over 70 at that time, indicating that the fish were of superior quality for freezing and storage.

The regression lines indicate that the refrozen samples prepared in May (Fig. 9, D; 8 weeks after capture) were somewhat poorer than those refrozen in March (Fig. 9 C; 7 days after capture). The range was 70–67 over 12–25 weeks storage for the former and 78–71 over 12–35 weeks storage for the latter. However, the quality of the samples given the lowest scores was still considerably better than acceptable.

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Initially (4 weeks after capture), thaw-drip values in samples prepared for chemical analyses (Table III) were relatively high for once-frozen Bank cod (see drip values of 4.3–13, Dyer et al., 1964b; and 4.4–10.5, MacCallum et al., 1964). The *p*H was low compared to values (MacCallum, unpublished data) of 6.7–6.8 for Grand Bank cod (once- and twice-frozen, first freezing before rigor) taken in February and in April 1965. The average total lipid was 0.94 ± 0.06 for 12 samples, similar to the value reported previously for trap-caught fish (MacCallum et al., 1964). The total amounts of free fatty acids averaged 164 mg/100 g muscle, equal to the value obtained earlier in trap cod and about three times that obtained in Newfoundland fall-caught, inshore cod (MacCallum et al., 1964).

TABLE III. Chemical assessment of once-and of twice-frozen fillets of Grand Bank cod taken in October 1964.

Sample[a]	Weeks in storage at −23 C after capture	Thaw drip[b] (% wet wt)	Total lipid[c] (% wet wt)	Free fatty acids[d] % total lipid	Free fatty acids[d] (*mg/100g*) muscle	*p*H
(1)	4	15.1	1.05	17.2	181	6.30
(2)		20.0	0.91	17.5	160	6.11
(3)		15.2	0.89	17.3	157	–
(4)		15.6	0.92	17.7	157	–
(1)	16	9.3	1.00	23.3	233	6.35
(2)		20.1	0.90	22.5	202	6.25
(3)		14.7	0.98	24.0	235	6.52
(4)		12.2	0.89	26.9	239	6.52
(1)	30	10.5	0.92	31.3	288	6.29
(2)		13.8	0.87	37.4	325	6.73
(3)		15.6	0.82	33.3	273	6.47
(4)		13.9	0.86	34.6	298	6.35
(1)	50	8.8	0.90	42.7	384	6.29
(2)		11.9	0.78	44.5	347	6.25
(3)		10.3	0.81	50.2	407	6.36
(4)		10.7	0.84	53.5	449	6.24

[a] Frozen once (1) before rigor; (2) in rigor. Frozen twice, first freezing (3) before rigor; (4) in rigor.

[b] For method see Dyer et al. (1962)

[c] For method see chloroform — methanol phase separation procedure (Bligh and Dyer, 1959).

[d] Free fatty acids were titrated against alcoholic NaOH and the results calculated for a fatty acid molecular weight of 300.

Production of free fatty acids through lipid hydrolysis held at the same rate for once- and twice-frozen samples throughout the time of storage at −23 C, the values for the two groups averaging 296 mg/100 g flesh after 30 weeks and 397 mg/100 g after 50 weeks. The *p*H of the samples was the same after storage for 4 and 50 weeks.

Though the first freezing of the twice-frozen samples was slow (Fig. 4), these samples were not lower in quality than the once-frozen fillets. This is supported by the taste panel scores at 3 weeks which in general are similar (Table IV). The result agrees with a previous assessment of the effect of freezing rate on the quality of frozen, stored trap-caught cod (MacCallum et al., 1965).

Initially, taste panel scores for once-frozen cod, although considerably lower than those given the 1962 Bank samples (Fig. 9), were comparable to those reported previously for Bank cod handled and frozen commercially (Dyer et al., 1962). Thereafter, as shown below, the quality of the October 1964 once-frozen samples deteriorated more rapidly than might have been expected from previous experience with frozen stored Bank cod.

TABLE IV. Average taste panel scores[a] and *p*H of once- and of twice-frozen fillets of Grand Bank cod taken in October 1964.

Sample[b]	Weeks in storage at −23 C after capture	Texture	Taste	Overall acceptability	*p*H[c]
(1)	3	74±1.0(21)	76±2.6(21)	73±3.1(21)	6.24
(2)		66±2.2(21)	78±2.5(21)	66±2.2(21)	6.13
(3)		80±2.5(21)	74±2.9(21)	78±2.1(21)	6.61
(4)		68±1.9(21)	79±1.2(21)	73±2.5(21)	6.40
(1)	16	66±4.1(23)	61±3.9(23)	65±4.1(21)	6.27
(2)		65±3.9(24)	71±2.0(24)	66±3.9(24)	6.33
(3)		51±2.9(23)	64±3.2(23)	55±2.1(23)	6.06
(4)		66±3.0(24)	68±2.1(24)	66±2.6(24)	6.68
(1)	30	39±2.7(28)	49±2.6(28)	42±2.2(28)	6.28
(2)		38±3.2(26)	42±3.4(26)	40±2.9(26)	6.09
(3)		54±2.0(26)	56±2.3(26)	54±1.9(26)	6.74
(4)		34±2.7(26)	48±3.2(26)	38±2.2(26)	6.42
(1)	50	41±2.1(24)	29±3.4(24)	33±3.8(25)	6.22
(2)		29±3.7(24)	34±3.2(24)	28±3.7(24)	6.08
(3)		46±4.2(24)	45±4.9(24)	45±4.4(24)	6.49
(4)		28±2.6(24)	36±3.2(24)	28±2.7(24)	6.39

[a] Scores are followed by the standard error of the mean and by the number of judgments (in parentheses).

[b] Frozen once (1) before rigor; (2) in rigor. Frozen twice, first freezing (3) before rigor; (4) in rigor.

[c] The *p*H was taken using the combination glass electrode on quickly thawed, uncooked portions.

Between 3 and 50 weeks storage, the quality of all once-and twice-frozen samples (Tables IV) dropped ($P < 0.05$). Scores for the once-frozen fish did not differ significantly between 3 and 16 weeks but those for the twice-frozen fish did. However, between 16 and 30 weeks, a halt to the downward trend occurred with the twice-frozen samples, pre rigor at first freezing. As a result, over the longer storage periods the latter samples maintained an acceptable, and the highest, level of quality.

The average *p*H values were not affected by storage. On the whole they were low. There appeared to be some correlation between texture scores and the hydrogen ion concentration (at thawing after each period of storage) in the case of the twice-frozen samples prepared from fish before rigor had developed. These samples tended to score higher, relatively, when *p*H was higher (3, 30, and 50 weeks after capture) and lower when *p*H was lower (16 weeks following capture).

After 30 weeks storage, three-quarters of all taste panel grades were considerably lower than those reported elsewhere in this paper for either Bank- or trap-caught cod. The October samples, regardless of treatment, exhibited chewy, hard, and rubbery characteristics. In addition, a characteristic granular or mealy texture, evident in a few once-and twice-frozen samples (freezing before and in rigor) 3 weeks after capture, was still about as prevalent in pre-rigor frozen samples at 30 weeks. At that time, too, a high proportion of all samples were slightly stale, stale, slightly sour or sour. After 50 weeks there was no evidence of granular texture. Toughening and poor taste were generally evident in the samples at 50 weeks, confirming the assessment at 30 weeks.

There was no observable effect of thawing and refreezing on the subsequent storage quality of the October catch. In fact, the twice-frozen samples, pre-rigor at first freezing, were superior as indicated above. For reasons not apparent other than because of the relatively high *p*H, the latter samples were better ($P = 0.05$) than once-frozen counterparts at all periods of storage with the exception of 16 weeks. This result, showing the maintenance of equal or better quality in stored refrozen cod than in the once-frozen product, is in agreement with the results (reported above) of storage tests of once- and twice-frozen July 19 catches of trap fish, but differs from the results obtained in 1961 (Dyer et al., 1962) and those reported earlier in the present paper for the other 1962 trap fish. In the two instances in which the storage quality of twice-frozen samples was not reduced from that of the once-frozen, the fish were of quite poor texture initially (in comparison with that of the June 27 trap fish and the March Grand Bank cod studied earlier.)

The reasons for the difference in quality between the March 1962 and the October 1964 cod are not known. Grand Bank cod spawn in April–June (Templeman, 1962), thus fish caught in March could very well be of good quality, as shown in the present instance, but so too should the catches taken in October at which time the cod should have recovered from spawning.

The studies on the March 1962 and on the October 1964 Bank cod focus attention on the variation in both initial and cold storage quality of cod taken in offshore waters. Some of this cod will be of excellent quality for freezing either once or twice, other catches will give only a fair to poor product when once frozen and stored and will rate with the poorest of the trap fish. Double freezing and restorage will do little to lower the quality further. However, as with trap-caught landings, the possibilities of twice freezing off shore catches appear to be good. The main point is the recognition that quality will vary with time and place of fishing.

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APPENDIX I

The previous seven papers on quality of cod were:

DYER, W. J., DORIS I. FRASER, D. G. ELLIS, D. R. IDLER, W. A. MACCALLUM, AND E. J. LAISHLEY. 1962. Quality changes in stored refrozen cod fillets. Suppl. au Bull. Inst. Int. Froid, An. 1962–1, p. 515–524.

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MACCALLUM, W. A., AND D. G. ELLIS. 1963. Water-thawing of frozen blocks of eviscerated, headed cod. Canadian Fish., **50**(12): 61–66. Also in Fish. News Int., **3**(1): 56–57, 1964; Fish Merchant and Processor, Jan/Feb., 1964; and World Fish., **13**: 56–59, 1964.

MACCALLUM, W. A., H. S. SHIEH, DOROTHY A. CHALKER, W. J. DYER, AND D. R. IDLER. 1964. Polyphosphate treatment of frozen cod. 2. Effect on drip, yield, lipid hydrolysis and protein extractability in twice-frozen Newfoundland summer trap and fall cod. J. Fish. Res. Bd. Canada, **21**: 539–548.

MACCALLUM, W. A. 1964. Temperatures and thaw-drip associated with electronic thawing of Newfoundland cod. J. Fish. Res. Bd. Canada, **21**: 719–726.

IDLER, D. R., W. A. MACCALLUM, DOROTHY A. CHALKER, AND J. T. LAUDER. 1964. Effect of environmental and/or physiological factors on quality. Newfoundland trap cod p. 98–100. *In* Rudolf Kreuzer [ed.] The Technology of Fish Utilization. Fishing News (Books) Ltd., London.

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NOTES

Deck Sampling of Research Vessel Catches

Population characteristics of a species may vary in space (both horizontally and vertically) and time. Reasonably complete descriptions of and explanations for these variations require widespread and repetitive research-vessel surveys, which reduce the bias toward large concentrations and large sizes introduced by commercial sampling. Detection of variation within the population may require that each research-vessel haul represent the population at the particular station occupied. This introduces two sampling problems: firstly, that of obtaining a random sample from the population at a particular point in space and time; secondly, that of subsampling if the catch is too large to examine in its entirety. The latter is considered here.

Apart from estimates of numbers and weight from each haul, the population characteristics usually of primary interest are the length and age distributions. The most effective way to determine the latter appears to be random selection from each length group, with subsequent adjustment on the basis of the catch length distribution (Ketchen, 1950; Pinhorn and Fleming, 1965). The problem then is to sample randomly the various length groups in the catch. This is not as straightforward as it may first seem since fish in the deck pound tend to be segregated by size (largest at the outer edges or top of the pile, smallest at the bottom). Procedures for handling to obtain random samples are described by Pope (1963) and Paloheimo and Dickie (1963). An alternative method, which reduces handling to a minimum, is described below.

Procedure. The method has been in use for deck sampling of large catches on vessels of the St. John's Biological Station for some years. It consists of sorting the catch, one species at a time, into groups of baskets and selecting one or more baskets at random from each group as it is filled. The ratio of sample to catch is chosen to provide at least 400–500 fish for measurement. All fish are measured in catches of this size or less. The baskets are chosen by placing a numbered wooden block on each and drawing one or more at random from a set of correspondingly numbered cards. The number of baskets selected will depend on the number of fish measurements desired. The number of baskets in each group will depend on the size of catch and available deck space but is usually 5–10.

Within this general outline two special practices are followed. Firstly, each group of baskets is filled simultaneously from the fish pound, or as nearly as it is possible to do this within the limits of available space. Secondly, those fish left over after the last full group has been disposed of are spread out into

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fractions of baskets over the remaining group; e.g. if the fish are being sampled from groups of 5 baskets, and if 1.5 baskets of fish remain at the end, these are distributed over the 5 baskets so that each will contain 0.3 basket of fish. At the same time an attempt is made to distribute these fish so that all sizes are represented in each basket. Again, this is accomplished by simultaneous filling, aided by rearranging fish between the baskets after the last part of the catch has been distributed among them. In fact this is always done for the last group of baskets, whether or not they are filled, since this group is likely to vary most in length distributions of individual baskets (Paloheimo and Dickie, 1963).

Application. Experiments to test the efficacy of the procedure were carried out on one large catch of redfish and two catches of cod.

The procedure for each experiment was as follows:

Experiment I. A catch of 116 baskets of redfish was handled as described above, the baskets being filled in 12 consecutive groups of 10 (the last 6 baskets were spread over 10 to give 10 times 0.6 basketfuls in this group).

Experiment II. A catch of 41 baskets of cod was measured in its entirety, but the baskets were not filled in predetermined groups and the measurements from the first 4 baskets were not separated. In order to use the data for present purposes, the measurements of the first 4 baskets plus one other basket were discarded, and the remainder numbered in the order in which they were filled on deck and treated as a catch of 4 groups of 9 baskets. Thus there was no spreading of the last part of the catch over the last group of baskets.

Experiment III. One-half of a cod catch of 54 baskets was measured (every second basket selected). Again there was no spreading of the last part of the catch. The baskets were numbered consecutively as they came from the pound and were treated as a catch of 3 groups of 9 baskets.

Experiments II and III were not strict empirical tests of the method. However, they do illustrate (following section) that selection of baskets from stratified basket lots gives reasonable results even with very small samples and without either simultaneous filling of basket groups or spreading of the last part of the catch. These two steps would provide extra insurance toward representative sampling. Thus, comparisons in these tests are of small samples with much larger ones, and may be regarded as analogous to experiment I, where the larger sample happened to be the whole catch.

For each sample one basket was drawn at random from each group by use of numbered cards and the sample length frequencies combined. Sample frequencies were adjusted to those for the total by dividing the frequency at each length class of the latter by the ratio of total fish measured to number in the sample. The redfish data were treated in 1-cm groups and the cod in 3-cm groups. Ten random samples were drawn for each experiment, with an additional ten 1-in-20 samples for the redfish data. Each of the latter was obtained by drawing at random one basket from each group of 20 for the first 100 baskets, 0.5 basket from the measurements of one of the next 10 baskets and $\frac{1}{2}$ of 0.6 (0.3 basket) from the last 10. The resultant frequencies were compared by means of the chi-square test for homogeneity, using 1-cm groups for redfish and 3-cm groups for cod. Cell frequencies containing 5 fish or less, at the ends of the distributions, were combined over several length groups. Two significant chi-square values occurred at P = .05, none at P = .01 (Table I).

As an illustration of the similarity of sample and catch length frequencies the number measured for samples 1–5 of each experiment was adjusted to the catch frequency by multiplying the number at each length by the reciprocal of the fraction sampled. The obvious features of each catch frequency, e.g. positions and relative heights of peaks, were generally satisfactorily reproduced in the adjusted samples (Fig. 1). Comparisons of average lengths and numbers of fish between the various samples and those actually measured (Table I) revealed few serious discrepancies. Sample average lengths were within 5% of the true figures, and numbers estimated from the samples within 10% of the actual numbers except for 2 of the 40 samples (numbers 7 and 10 of experiment III).

Discussion and conclusions. While more data for the same and other species would be desirable, the present results were considered of sufficient interest for presentation. We had originally planned to sample various numbers of baskets from each group, comparing the resulting length frequencies with that of the total measured to determine the level of sampling necessary to give adequate representation of the catch length distribution. In fact, on the basis of the chi-square tests (Table I), it was apparent that samples of just one basket from each group were usually satisfactory.

Previous authors (Pope, 1963; Paloheimo and Dickie, 1963) have emphasized that basket-to-basket variability of fish lengths in research vessel catches makes it extremely difficult to select random samples without some special treatment of the catch, sorting of species being a first requirement. Pope (1963) further suggests a procedure to obtain fish from the top, middle, and bottom of each basket caught. Paloheimo and Dickie (1963) recommend mixing of fish from baskets into a lesser number of larger boxes, some fish from each basket in the catch being transferred to each box. The boxes are then used as sampling units. Both procedures require that the catch be handled twice before any biological examination begins, and this becomes formidable for very large catches. The procedure suggested here adds very little in time and effort to the job of clearing away the catch.

An analogous, though not entirely similar, procedure to that described here was tried by Paloheimo and Dickie (1963). Catches were stratified into basket groups according to the order of filling and measurements of fish in each basket recorded separately. The heterogeneity within basket groups was much less than between them, though departure from randomness was significant even within groups. A major proportion of this heterogeneity was introduced by the last group of baskets (because of small fish in the last one or two of the group). This within-group heterogeneity would undoubtedly be reduced by our procedure of simultaneous filling combined with spreading of the last group. In any case, too much emphasis may perhaps be placed on basket-to-basket

TABLE I. Values of χ^2 from homogeneity tests, with average lengths (cm) and numbers of fish for samples and measured totals. Number in each sample was multiplied by the reciprocal of the fraction sampled. Significant χ^2 values (.05 level) are indicated by an asterisk.

Exp No.	Species	Fraction sampled		Measured catch	Sample										Range of differences (%)
					1	2	3	4	5	6	7	8	9	10	
Ia	Redfish	1/10	χ^2	...	28.7	15.2	25.6	21.7	41.2*	14.8	31.8	21.8	19.5	16.5	...
			Avg length	34.6	34.4	34.9	34.2	35.0	34.5	34.6	34.3	34.2	34.6	34.6	−1.2 to 1.2
			No. fish	5890	5930	5650	6200	5690	6080	5930	6000	6130	5820	5990	−4.1 to 5.3
Ib	Redfish	1/20	χ^2	...	13.6	28.4	23.7	32.9	28.3	18.8	31.0	16.4	19.8	25.1	...
			Avg length	34.6	34.5	34.1	34.1	33.6	35.4	34.8	33.8	35.2	34.4	35.3	−2.9 to 2.3
			No. fish	5890	5880	6160	6120	6180	5440	5880	5980	5840	6000	5780	−7.6 to 4.9
II	Cod	1/9	χ^2	...	13.4	20.0	19.1	8.1	13.1	9.0	13.9	10.8	11.2	10.9	...
			Avg length	57.5	55.2	57.6	59.5	56.6	58.6	58.0	57.2	57.7	57.2	57.7	−4.0 to 3.5
			No. fish	753	828	747	720	819	711	738	720	774	765	738	−5.6 to 10.0
III	Cod	1/9	χ^2	...	23.7	14.3	13.5	5.0	19.5	27.1*	18.1	8.3	18.4	20.2	...
			Avg length	54.8	53.9	55.5	55.1	54.5	54.5	55.0	55.9	54.3	54.4	55.5	−1.6 to 2.0
			No. fish	671	639	639	693	702	729	639	594	693	702	585	−12.8 to 8.6

Redfish: df = 24, χ^2 at .05 = 36.4
χ^2 at .01 = 43.0

Cod: df = 15, χ^2 at .05 = 25.0
χ^2 at .01 = 30.6

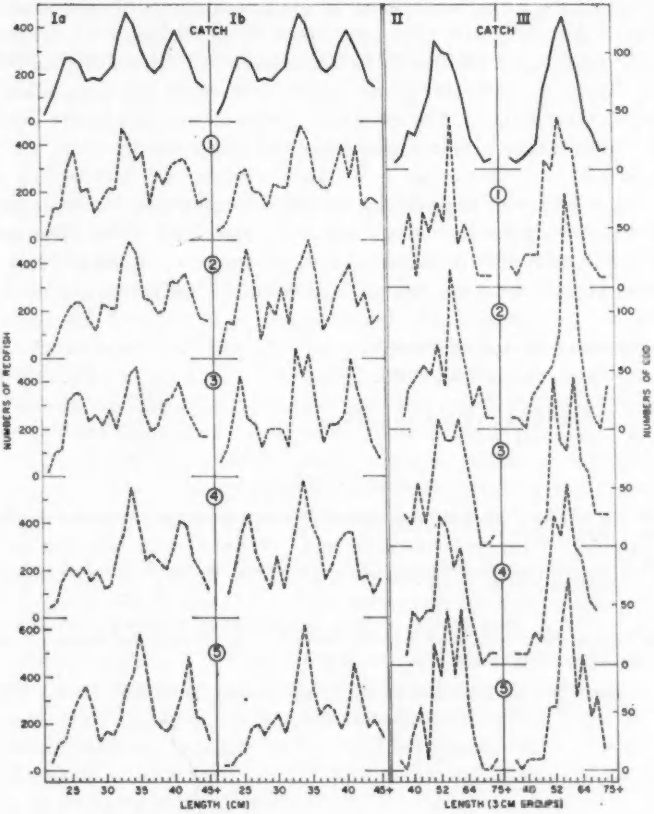

FIG. 1. Length frequencies of total fish measured and (adjusted) of five random samples of one-tenth (Ia) and one-twentieth (Ib) for the same catch of redfish, and one-ninth for two catches of cod (II and III). Average lengths and numbers of fish are given in Table I.

variability. It is not important that the size distribution in any one basket be similar to that of any other, but rather that baskets be selected so that their combined size distribution is a random sample of that of the catch. Simultaneous filling of basket groups plus spreading of the last group apparently reduces within-group heterogeneity sufficiently to accomplish this, even with the very small samples presented here. In fact it was accomplished in the cod experiments without rigid adherence to the usual procedure.

It must be emphasized that in our usual deck sampling much larger cod samples than those used here would be drawn, thus eliminating much of the "raggedness" in the length distributions (Fig. 1). In fact the results indicate that an order of sample size of about one-tenth of the catch should be considered a minimum for each species. Selection of larger samples may be performed without extra effort in handling, and the time taken for additional fish measurements is relatively little when compared to that necessary for sorting and sampling the catch. More complex sampling procedures could perhaps allow sample sizes to be further reduced, but available time and manpower are better spent in examination rather than excessive handling of the catch.

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A Portable Press for Making Impressions of Salmon Scales

Ages of Pacific salmon (genus *Oncorhynchus*) are commonly estimated by examination of scales. The outer surface of a scale bears a series of circular corrugations (circuli or sclerites) confined mainly to the anterior part of the scale. Presumably reflecting alternations of periods of rapid and slow growth during summer and winter, respectively, the circuli are alternately grouped close together (to form so-called "annuli" or "winter" bands) and more widely spaced ("summer" bands). A number of methods of mounting the scales for examination have been used over the years. Mounting of the scales in various media (such as water glass) was a standard procedure until recently when the common method used by salmon workers has been the making of impressions of the scale surface on heated sheets of cellulose acetate (e.g. see Clutter and Whitesel, 1956). Such impressions provide clearer definition of the circular ridges than can be provided by microscopic examination of the original scale because the original scale tends to be quite opaque and the circuli are often obscured by pigmentation. Making of impressions also permits production of copies of scales for exchange with other agencies and facilitates permanent storage (original scales mounted on slides often become brittle and unreadable with age). The scale impression device commonly used in the laboratory is a large, heavy machine which is basically a large hydraulic jack and thermostatically heated plates in which the scales are placed for impression. Because of its large size and weight, this machine must remain fixed in one location. Thus, if on-the-spot examination of scales in the field is required the scales themselves must be examined or sent in to the laboratory for impressing which results in some delay in analyses of the raw data.

A variety of techniques may be used for making scale impressions in the field, including squeezing with a jeweller's press, or using almost any type of simple pressure device. The present paper describes a portable press (Fig. 1) capable of producing in the field high-quality impressions of 50 scales at a time.

Essentially, the portable scale press is the same as the larger scale press in the laboratory. It contains one thermostatically-controlled plate, and pressure is applied by tightening the plates using a type of screw usually used in a bench vise. The portable press is of simple construction, and does not entail the need for any casting. A moderately equipped machine shop can produce one in a few days, with the basic elements that are required for its construction. The overall dimensions of the portable scale press are $9 \times 7 \times 5\frac{1}{2}$ inches, the total weight is about 4 lb, and the electrical power required is 110–120 v.

Description of parts and the construction. The parts and instructions for the construction of the portable press are listed below. Plans outlining the dimensions of the various components are given in Fig. 2.

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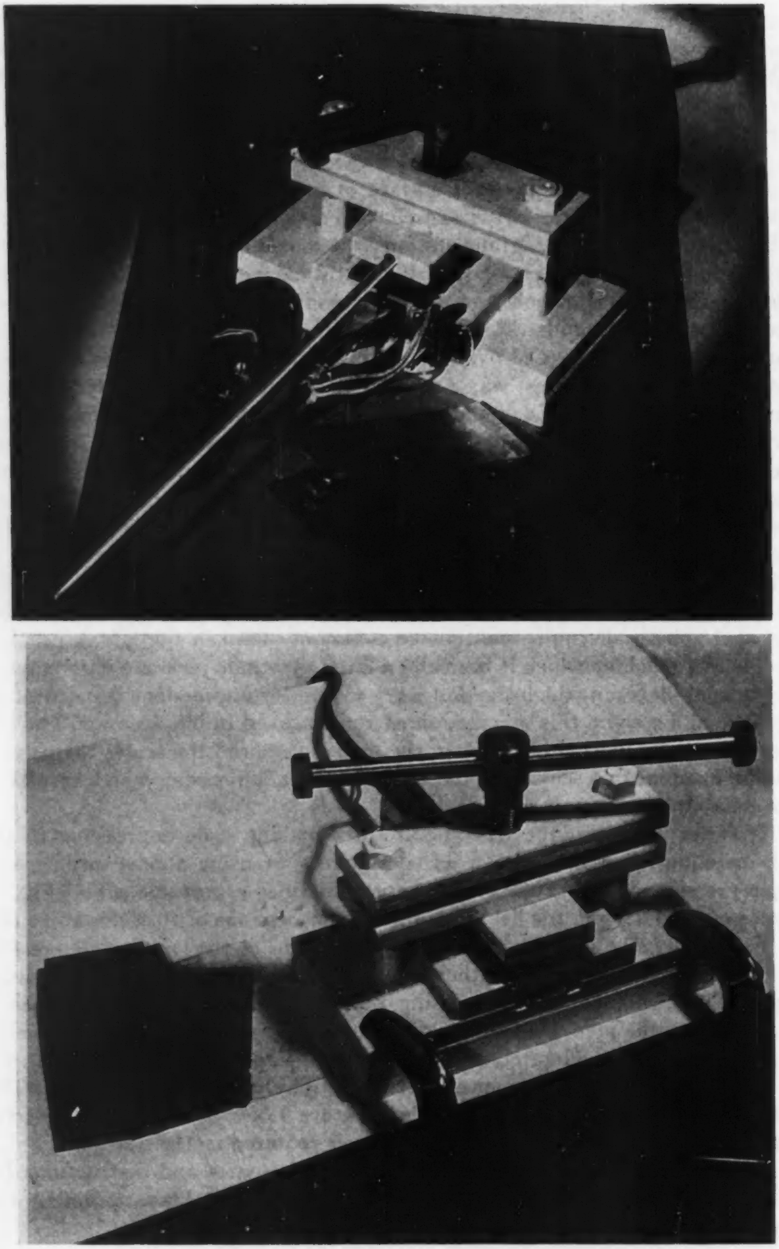
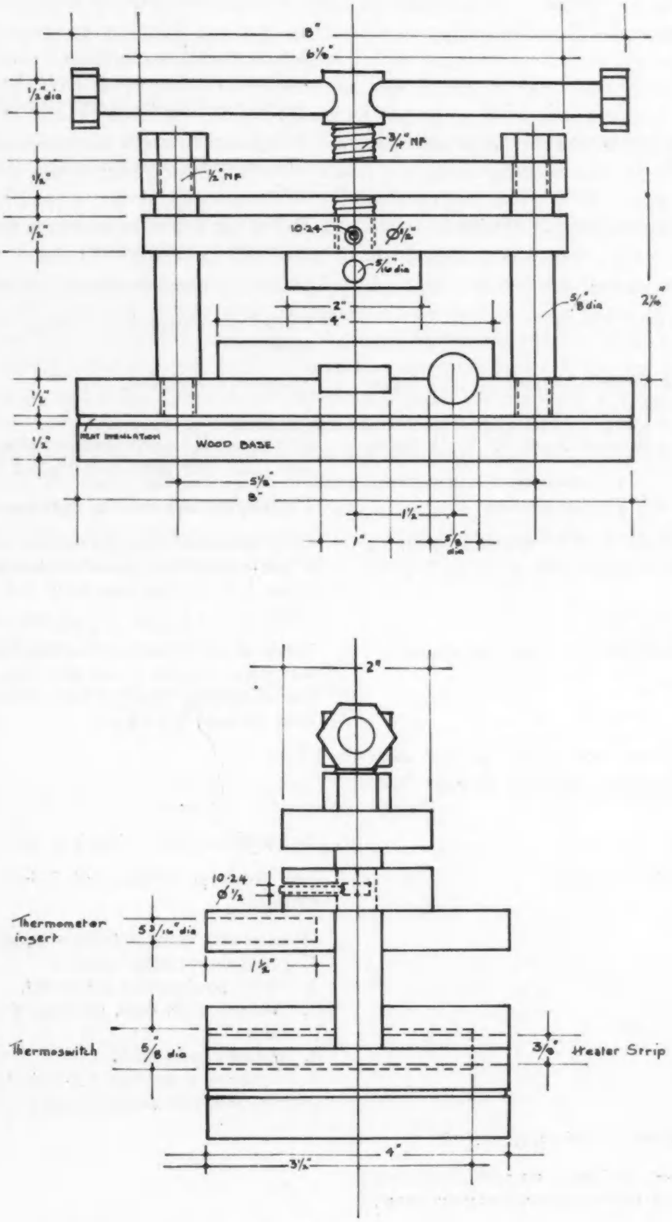


FIG. 1. A portable press for making impressions of salmon scales.


8"
6⅝"
½" dia
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½" NF
½"
10·24
Ø½"
5/16" dia
2"
4"
5/8" dia
2⅝"
½"
½"
HEAT INSULATION
WOOD BASE
5⅝"
8"
1½"
1"
5/8" dia
2"
10·24
Ø½
Thermometer insert
5/16" dia
1½"
Thermoswitch
5/8 dia
3/8" Heater Strip
4"
3½"

FIG. 2. Plans outlining dimensions of the various components for the portable press.

Part	*Construction detail*
Base-plate 8 × 4 × ½ inches steel plate	Drilled and threaded for pressure plate screws. Drilled and threaded for two columns and four wood screws to secure base. Channelled for heater strip, and for thermoregulator.
Lower pressure plate 4 × 4 × ½ inches steel plate	Drilled to take screws to secure to base plate. Channelled for heater strip and thermoregulator.
Upper pressure plate 4 × 2 inches	Drilled for screws to secure to cross guide plate and for thermometer.
Cross guide plate 6½ × 2 inches	Drilled for columns and centre screws.
Top plate 6½ × 2 inches	Drilled and threaded for columns and centre screw.
Two vertical guide columns 3¾ × ½ inches diameter steel	Threaded at each end.
One centre pressure screw 3¼ × 1½ inches diameter	(16 thread to 1 inch) Mounted at upper end with 1 × 1 inch diam collar drilled for lever.
One lever 9 × ½ inches diameter	Threaded at each end for retaining nut.
One strip heater 7 × ¼ × ⅜ inches having 3½ inches heating length	(Both terminals) Mounted between base-plate at one end and lower pressure plate centrally. Type: 1 × 7 inch type SNH 115 v up to 1200 F.
One thermoregulator 3 × ⅝ inches diam.	Mounted between base-plate and lower pressure plate 1½ inches to one side of heater strip (centre-centre). Type: ⅝ inch diam flange head, 10 amp, 115 v a-c.
Base-board 8 × 4 × ½ inches ply with sheet asbestos heat insulation between base-board and base plate	
Two bench clamps	To hold press rigid on bench or table.
Sandwich 5½ × 2½ inches	Two ferrotype plates, and ¼ inch rubber cushion. Order of each layer as follows: Upper — 1. ¼-inch sheet rubber cushion. 2. Ferrotype plate face downward. 3. Gummed scale card, gummed side downward. 4. Acetate plastic sheet. 5. Ferrotype plate face upward. ((5) rests directly on heated pressure plate.)
Flexible extension, 2-pin plug, 110–120 v	
Metal cover lined with electrical insulating material to cover terminals of strip heater.	

Method of mounting scales and making the impressions. Scales are mounted with the corrugated outer surface upwards on heavy gummed paper cards (2½ inches wide by 4¾ inches long). Using cellulose tape, these cards are attached, with gummed side inward, to plastic acetate cards of the same dimensions. When the temperature of the top plate of the scale press has reached 110 C, and the heater has been adjusted to maintain this temperature, the scale card (gummed paper card plus the acetate card) is placed between two 2½ × 5-inch ferrotype plates which are backed with ⅛-inch rubber sheet of the same dimensions and inserted between the upper and lower plates of the press. The plates are then closed by tightening the screw to the desired pressure required to make clearly defined impressions of the scales. As there is no pressure gauge some experimentation will be required to obtain the most suitable impressions. We have found that sufficient pressure is obtained by turning the lever one-quarter to one-half turn beyond the point at which pressure is first felt by the operator. The card should remain under pressure in the press for a period of time ranging from ½ to 1 min. As soon as the card has been removed from the press, the gummed card should be separated from the acetate card to prevent any possibility of the two sticking together. The acetate cards should then be placed under a weight, so they will retain their flat shape after they have cooled.

The surface of the ferrotype plates should be cleaned frequently to obtain clear impressions. This can be done with acetone applied with a clean rag.

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Pigments in the Belly Skin of Coho Salmon (*Oncorhynchus kisutch*)

During an investigation of the parr-smolt transformation of coho salmon (*Oncorhynchus kisutch*), it was observed that as many as five spots exhibiting fluorescence or absorbance under ultraviolet radiation were present on chromatograms of skin extracts. Since the number of these spots appeared to depend on the physiological state of the animal it was decided to investigate them more fully.

Presmolt coho (yearling coho prior to acquiring visible smolt characteristics) were collected in Hunt Creek, a tributary of the Big Qualicum River. Migrating smolts were obtained near the mouth of the Big Qualicum River and prepuberal coho (grilse) which had spent a year at sea were caught in the Strait of Georgia. Coho fry were obtained from fish hatcheries in Washington State and reared in captivity at this laboratory. All samples were anaesthetized, frozen, and stored at –20 C. A rectangular section of skin (Fig. 1) was removed from partially thawed fish for analyses and its area measured. As crystals, which were probably guanine, were present between the skin and muscle and would not be included if the skin only was used, a thin layer of muscle was included with the skin. No fluorescing or absorbing spots were visible on chromatograms of extracts from a deeper, thin layer of muscle.

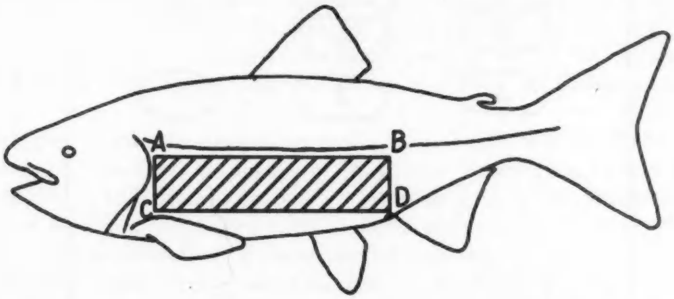

FIG. 1. Sketch of salmon showing rectangular skin area which was removed for extraction of pigments. The upper limit A–B was approximately two scale widths below the lateral line. Point C was above the insertion of the pectoral fin, and D above the anus.

The skin was digested in N KOH for 24 hr at room temperature and the volume adjusted to give 125 mm^2 of skin per milliliter of final solution. The solutions were filtered through glass wool, stored at 2 C and analysed within 2 days; 100 λ of each solution were applied to Whatman #40 paper and the pigments were separated by descending chromatography at room temperature

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for 24 hr using isopropanol, HCl, H_2O (170 ml isopropanol; 41 ml concd HCl; made up to 250 ml with H_2O) (3). The papers were dried at room temperature and examined under long wave (366.0 mμ) and short wave (253.7 mμ) ultraviolet radiation.[1] A typical chromatogram is shown in Fig. 2. Pigments 1, 2, and 3 appeared as absorbing spots under short wave UV. Pigments 4 and 5 were not visible at this wavelength, but appeared as light-blue fluorescent spots under long wave UV. The intensity of spots 1 and 2 appeared to be high in

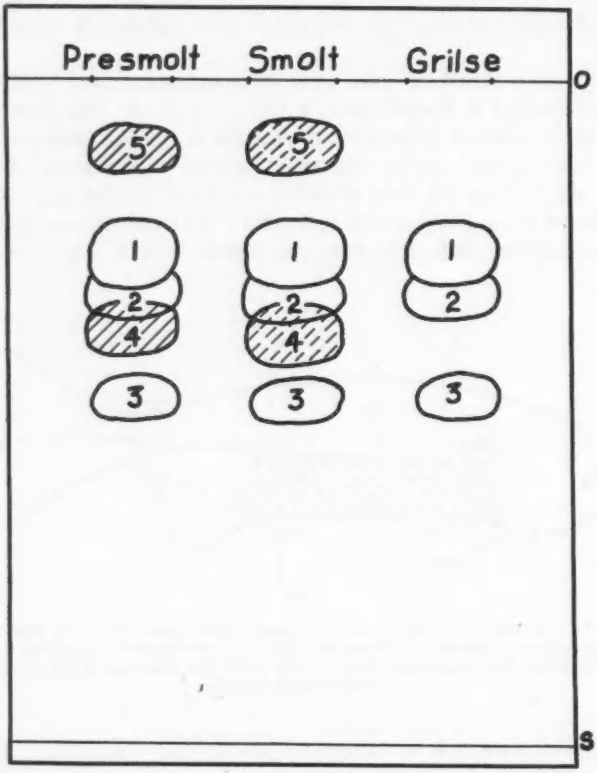

FIG. 2. Chromatogram of extracts of belly skin from a premigratory coho yearling (presmolt), a migrating coho yearling (smolt), both taken from fresh water, and a prepuberal 2-year-old coho (grilse) from sea water. 1, Guanine; 2, hypoxanthine; 3, "guanylic acid"; 4 and 5, "pterins." The blue color of guanine masked the purple color of hypoxanthine where the two fractions were admixed. *Open areas*: absorbing spots under shortwave radiation; *Solid bars*: light-blue fluorescent spots under longwave radiation; *Broken bars*: traces of light-blue fluorescent spots under longwave radiation; O — origin; S — solvent front.

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[1] Ultra Violet Products Inc., San Gabriel, Calif. (Short wave U.V. Model No. C81; Long wave U.V. Model No. XX15X).

smolts and grilse but low in presmolts. The concentration of pigment number 3 appeared to be similar at all three stages. The intensity of spots 4 and 5 was high in presmolts and low in smolts. Pigments 4 and 5 were not detectable in grilse. Chromatograms from 3- and 10-month-old hatchery-reared coho appeared identical to those from wild presmolts and chromatograms from hatchery-reared smolts appeared identical to those of wild migrating smolts.

For identification, chromatograms were run for 48 hr to further separate the spots. The areas corresponding to individual spots from many papers were pooled, eluted with 1% NH_4OH, filtered, evaporated to dryness and dissolved in 1% NH_4OH. Spots 1–5 were designated fractions 1–5 and rechromatographed using *n*-propanol–1% NH_4OH (2:1) (2). This procedure removed traces of fraction 2 from fractions 1 and 4. Eluants from all five fractions were then chromatographed twice more by this procedure.

From absorption spectra, absorption ratios, fluorescent spectra, and comparison with pure compounds, fraction 1 was identified as guanine and fraction 2 as hypoxanthine. Fraction 3 was tentatively identified as guanylic acid. Fraction 4 was shown to contain a mixture of two components, 4a and 4b, when chromatographed with *n*-propanol–1% NH_4OH. Not enough material was available for absorption spectra determinations, but at *p*H 11 components 4a and 4b exhibited maximum fluorescence at an excitation of 365 mμ. At this excitation the fluorescent maximum of component 4a was at 430 mμ and that of 4b at 435 mμ. Not enough material of fraction 5 was available for spectral analysis.

From the fluorescent characteristics it is probable that fractions 4a and 4b are pterins. Fraction 5, because of its similar appearance to fractions 4a and 4b under UV radiation, is probably a pterin as well. It has been shown that the skins of amphibia and fishes contain pterin pigments and in several species the types and concentration of these pigments change during metamorphosis (1, 4). Further work on the identification of fractions 4a, 4b, and 5 is in progress.

We are indebted to Messrs D. B. Lister and R. A. L. Harvey, Department of Fisheries of Canada, Vancouver, B.C., for obtaining the wild presmolts and migrating smolt samples, to Dr G. R. Bell and Mr S. C. Devereux, Fisheries Research Board of Canada, Nanaimo, B.C., for the grilse samples, and to Messrs G. Watkins and L. Esary, Superintendents of the Samish and Skykomish Hatcheries, respectively, State of Washington Department of Fisheries, for the hatchery-reared fish.

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Development of the Anal Fin of the Capelin

Hart (1937) pointed out that anal fin rays 7–10 of the mature capelin, *Mallotus villosus* (Müller), were enlarged at their distal ends so as to practically eliminate the intervening membrane. He also referred to the fusion of the ends of some of these rays. Vladykov (1941) gave a detailed description of this fin in the mature male and female of this species. He divided the anal fin of the male into three sections (Fig. 1): (1) the "anterior section" with 9 or 10 free rays, (2) the "middle section" with 4–6 rays fused at their distal ends to form a plate which he called the lamina pinnae ani, and (3) the "posterior section" made up of 9 or 10 free rays. In this paper the development of the anal fin in immature, maturing, and mature male capelin is traced and compared with that of the female.

Except for the mature fish (Fig. 2b, h), the material was collected during offshore research cruises by vessels operated by the St. John's Biological Station. The material was stored in 5–10% formalin before staining. After most of the flesh was removed from the base of the fins they were placed in a weak solution of Alizarin Red for 5–10 hr. Following this they were washed with running tap water for about 24 hr, dipped briefly in a solution of approximately 1% potassium hydroxide, washed again, and cleared with glycerine.

Most capelin spawn at age 3 (Templeman, 1948; Pitt, 1958) during June and July and the spawning form is well known. Immature capelin are only occasionally caught near the coast and are not recognized as capelin by local residents. After spawning most of the males and probably a large proportion of the females die.

Vladykov's (1941) figures of the anal fin were drawings and show the lamina pinnae ani as a continuous structure. In the material studied by the author the distal ends of the middle rays were definitely fused, but in the photographs (Fig. 1, 2h) the original separation between the rays appeared as a thin line.

Very little change occurred in the anal fin of the maturing female (Fig. 2a, b) but there was a gradual change in the anal fin of the male as the fish became mature. About 1 year before spawning this fin was similar to that of the female (Fig. 2a, b, c). By August of the year preceding spawning the rays of the anal fin of the male had grown in length and thickness (Fig. 2d). This widening increased as illustrated by the November specimens (Fig. 2e) so that by March (of the spawning year) the middle section was differentiated by the dichotomous splitting of the distal ends of the middle 4–6 rays (Fig. 2f). By May (Fig. 2g) the widening of the rays was nearly completed with the enlarged tips of the rays of the middle section forming the lamina pinnae ani which was completed at the spawning time (Fig. 2h).

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The enlarged anal fin of the male apparently aids in making a hollow in the sand where the eggs are partially buried, and after spawning this fin is considerably damaged (Templeman, 1948).

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(Fig. 1 and 2 *opposite*)

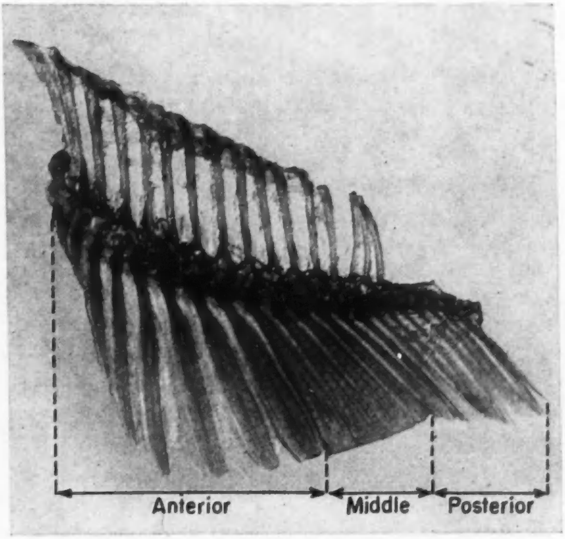
Anterior
Middle
Posterior

FIG. 1. Photograph of the typical anal fin of a spawning male capelin from a specimen caught near St. John's, Nfld., June 24, 1965.

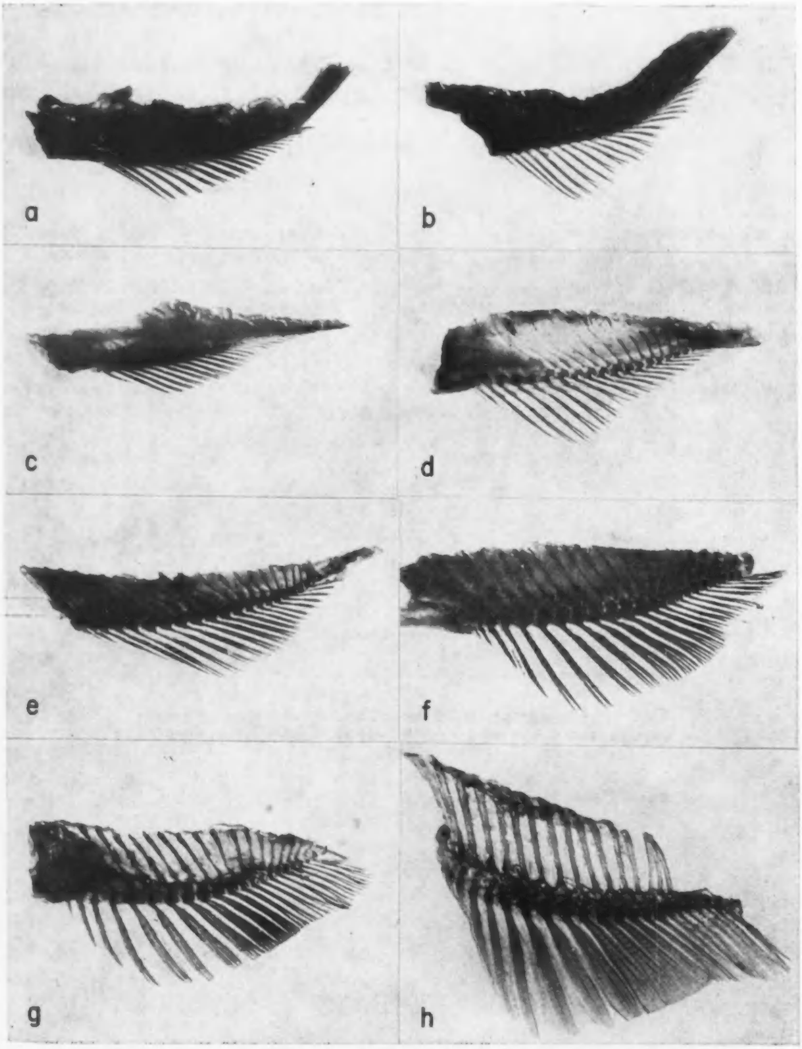

FIG. 2. Photographs of dissected and stained anal fins of capelin showing also (in d–h) the interspinous bones. (a) Immature female, length 141 mm, August 1953; (b) spawning female, length 168 mm, June 1955; (c) immature male, length 117 mm, May 1951; (d) immature male, length 145 mm, August 1951; (e) immature male, length 160 mm, November 1950; (f) maturing male, length 164 mm, March 1951; (g) maturing male, length 170 mm, May 1953; and (h) spawning male, length 185 mm, June 1955.

Incidental Records on Behaviour of Eels in Lake Ainslie, Nova Scotia

Canadian east-coast researchers and industrialists have a lively current interest in eels and have been soliciting information. Hence the preparation of this paper which is based on incidental records made in the course of the Fisheries Research Board's 1935 Atlantic Salmon Investigation in the Margaree River system, N. S.

My assignment in this investigation, under Dr A. G. Huntsman, was to make observations on fish and on water conditions at two freshwater hydrographic stations three times each weekday for 3 months (June–August). Most of this work was done from a punt, but considerable time was also spent on the bridge at Scotsville. This was a particularly good vantage point for watching fish, for fishing, and for talking to local fishermen about fish, including eels. Eels were important as food fish in Scotsville and adjacent areas of Cape Breton Island and habits of eels were well known to local fishermen.

One of the hydrographic stations was in the northern end of Lake Ainslie (45°11′N lat; 61°10′W long) near its outflow into the Southwest Margaree River. The other was 2 km (1.2 miles) to the north and in the middle of the river at Scotsville highway bridge.

This report is based on my frequent observations at and near the hydrographic stations and on fishermen's reports. The North American eel literature consulted so far describes none of the behaviour characteristics reported here except migration and very little on eel migrations in eastern Canada.

Lolling on weed. Eels were rarely seen at the river station in June and early July when the water was cool and high. But anglers did catch them occasionally on baited hooks while fishing from the bridge in the evenings. As the season advanced, eels were seen there more frequently and fishermen caught them more regularly. On July 27, for example, one angler's evening catch was five eels including one yellow-bellied (50 cm long) and four white-bellied (45–50 cm). At that time I did not appreciate the significance of these two colours — white (silver) characterizing mature eels ready for seaward migration and yellow characterizing freshwater residents (Vladykov, 1955).

Similarly, eels were rarely seen early in the season in the upper river. But by mid July pond weeds and leaf-stems of water lilies at the edges of the channel had grown long and lay almost horizontally either near or at the surface of the slow stream. With this change, eel sightings became more and more common. Many eels lay motionless, looped in inverted "U"s over stems of these water plants. Sometimes the eels lay on weeds that were as much as 1 m below the surface but more often they were close to or actually at the surface where their bellies glistened in the sun. By the end of July great numbers of these lolling fish were regularly seen on trips between hydrographic stations. But by late August sightings were less common.

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On August 18, two lolling eels were observed in precisely the same positions on the same weeds at 2:00 PM and 7:00 PM. They were presumed to be the same fish. Fishermen stated that such observations were common in midsummer. They regarded them as sleeping.

With this change in eel habits, many fishermen quit angling and took up summer spearing (Day, 1948) "on the weed." They were very successful because they were able to approach the fish to within spearing distance (about 2 m, 6–7 ft) by floating downstream in punts. When disturbed, surface-lolling eels splashed loudly and disappeared with lightning speed.

Eels caught in June and early July were dry (not fat) when cooked. Those taken on the weed after mid July seemed fatter and were more relished by the local people.

Eel balls on bottom. Early in the season eels were rarely seen in the outflow which was near the lake hydrographic station. At that time the outflow was relatively free of water plants. But by late July dense beds of rushes (*Scirpus*) had grown up forming a vast wilderness of quiet shallows and *single* eels were a common sight.

On August 17 three spherical clumps of eels (diameters about 0.5 m) were sighted about 25 m apart on the bottom in the outflow. The estimated numbers of eels in these clumps were 15, 20, and 30. The fish were knotted together in tight, complicated, motionless masses clustered about the bases of rushes at a depth of about 1.5 m (5 ft). A young fisherman had come in the punt that day to spear eels. He had no chance to make more than a single thrust at any one clump because the disturbed fish immediately disentangled themselves and shot out like arrows in all directions. Nevertheless, he caught as many as three eels per thrust.

Free-floating balls. Several experienced fishermen stated that they had seen similar stationary clumps, which they called "eel balls," on bottom and two fishermen described sightings of free-floating balls a number of years previous. None was seen in 1935. Both sightings were in deep water offshore at about the same time of year—just preceding late-summer downriver migrations. These balls were described as bobbing up and down slowly, sometimes near the surface, and sometimes actually breaking the surface.

The first sighting was in 1914 by Mr Malcolm John MacPhail, who was a crewman that year on a small Lake Ainslie steamer. This ball was seen about half a mile (1 km) off Kenlock in Loch Ban, a bay on the west side of the lake. It was about 6 ft (2 m) in diameter—"big enough to fill two barrels." Flipping tails on the outside of the ball apparently caused a slow rolling.

The second sighting was by Mr Peter C. MacKay, who operated an eel weir (Day, 1948) at Scotsville. He could not remember the year but he described covering the bottom of his punt with eels by using a dip net (diameter 0.5 m) to scoop up the "best part" of a large mass of eels in lower Lake Ainslie. As he recalled it, this mass was shaped more like a vertical cylinder than a ball. From his account it seems that eels in these larger balls disperse more slowly when disturbed than those in the small bottom balls seen in the outflow on August 17, 1935.

Another fisherman ventured the opinion that free-floating balls at the surface might be the basis for the traditional "great beast of Lake Ainslie." This monster was known to the Gaelic-speaking of the community as "beathach mor Loch Anaslaigh." Tales of it were common among the older people.

Dr D. E. McAllister of the National Museum of Canada has drawn attention to the statement by Fries et al. (1893, p. 1031): "Frequently the migrating eels knot themselves together in bunches, and large bundles, often a fathom in circumference, are seen lying in the lakes or trundling down the streams." This applies to the European eel, *Anguilla anguilla* (Linnaeus) 1785, which is referred to by Fries et al. as *A. vulgaris*. Thus it appears that both North American and European eels form on-bottom resting balls and free-floating balls.

Seasonal migration. In the St. Lawrence River system downriver migrations of mature (nonfeeding) eels usually occur from August to November (Vladykov, 1955). But the fact that in August (Fig. 1) at least some of the eels from MacKay's weir were feeding suggests that they were part of a composite run of immature (feeding) as well as of mature (nonfeeding) eels. Furthermore, MacKay stated that his catches were often mixtures of white-bellied and yellow-bellied eels. My colleagues, Drs P. F. Elson (personal communication, 1966) and Smith and Saunders (1955), state that it is common for eels of all sizes to run together. Elson observed this in 1941 in Goldmine Brook, Guysborough County, N. S., during summer freshets. The eels were 10–55 cm long. The apparent composite nature of the Lake Ainslie run is therefore not unusual.

The local fisheries officer, Mr R. W. Watts, reports (personal communication, 1966) that the Southwest Margaree eel weir fishery is still active during August and September. However, in 1935 MacKay began catching eels in his weir on July 7. He also made hauls on July 21 and 22 but considered them hardly worthwhile (Fig. 1). His good catches began in August. On August 20 he caught only five eels. On August 23 he got 600 and considered this a good catch. This was a dark, rainy night and the run lasted from 8:00 pm until midnight, then stopped suddenly. On August 24 he took 800. No weir catch records beyond this date for 1935 are available.

MacKay kept a diary of his weir catches. His records for the years 1925–35 are shown in Fig. 1 which includes moon phases. As a boy MacKay remembered helping his father at the weir in autumn when there were 4 inches of snow on the ground. His impression was that there had been a tendency within his lifetime for eel runs to begin earlier in the year and to end earlier. Figure 1 suggests that MacKay may have been right. But his records may be variously interpreted.

MacKay stated that, as a rule, eels begin their downriver migrations in late July or August in the "dark of the moon," i.e. when there is no moon showing between sunset and midnight. This seems to be borne out by Fig. 1.

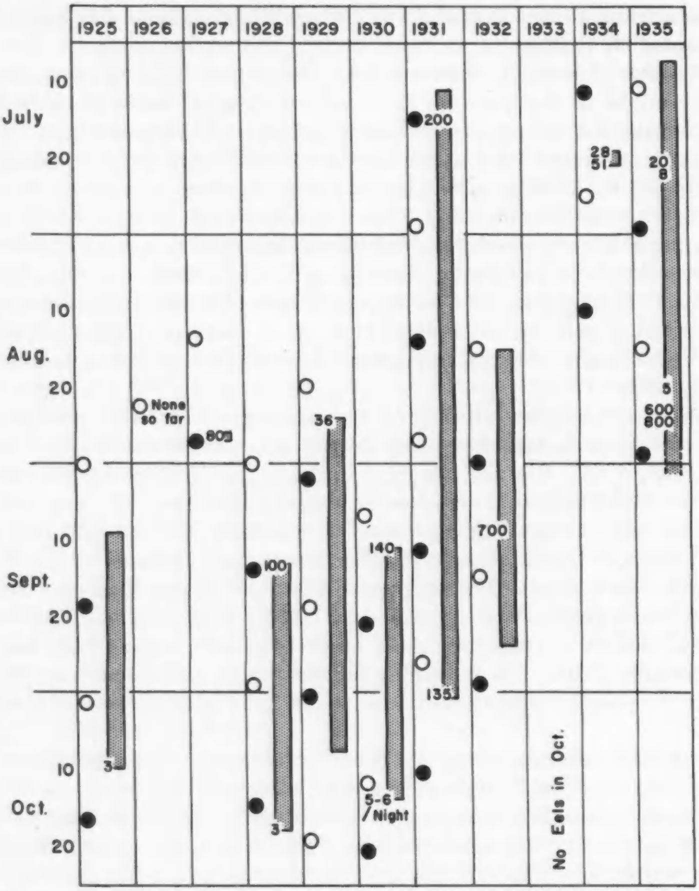

FIG. 1. Summary of MacKay's records of weir-catches of eels below Scotsville. Numbers in figure are counts of eels taken during nightly sets of the weir net. Bars cover period during which runs were recorded. Solid dots indicate new moon and open dots, full moon.

He also stated that the best runs took place on dark, rainy nights, and this agrees with observations by Smith and Saunders (1955).

However, no such support has been found for certain other statements MacKay made about migrations—that in years with few dark, rainy nights before the end of September, there may be no eel runs because thereafter the water is too cold; that late in the season he usually caught three or four very large eels, about 4 ft (120 cm) long, and that their arrival marked the end of the season's fishing; that in any particular evening's fishing, yellow-bellied eels entered the weir early in the run and white-bellied eels later.

Other investigators may have opportunity to check some of these generalizations.

Sound production. On calm evenings after dark, in mid August, minute, almost bird-like chirping or sucking noises were often heard at the Scotsville bridge river station. These were like the sounds one makes in his cheek when trying to dislodge something stuck in a tooth. On inquiry, fishermen stated that migrating eels were responsible for these sounds and that they were feeding noises—not "voices." This was the season when both eels and gaspereau fry were moving downriver from the lake. And guts of eels caught at this time by anglers and in MacKay's weir often contained a silvery slime which was interpreted as remains of young gaspereau.

On August 23 eels in the bottom of MacKay's punt were heard making precisely these same chirping sounds after they were taken from his weir, ½ mile (1 km) below Scotsville. It was not determined how they made these noises. But they also made sounds like gas bubbles moving in a person's intestine. These noises seemed to be caused by air escaping from the operculum but this could not be verified because the lantern light was poor. This latter sound was not heard in nature.

It is doubtful that either of these sounds is specifically related to migration. It may be that eels can chirp at any time during their active season. However, chirping frequencies may vary with local abundance of eels under some conditions and for this reason might be useful to eel weir fishermen as indicators of when to set their nets.

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